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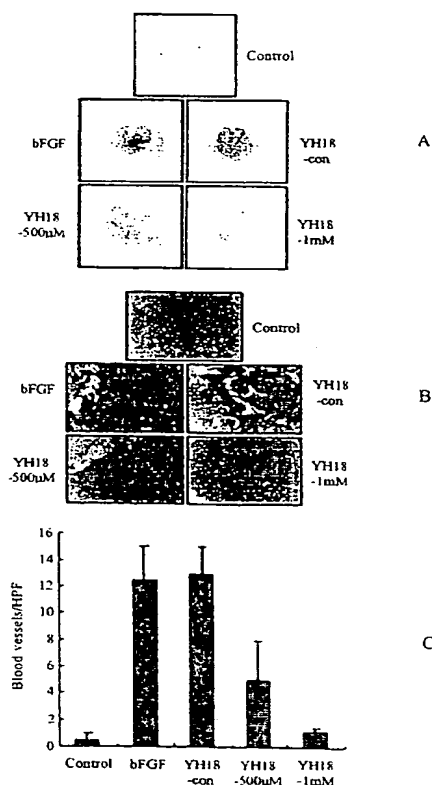
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(54) Title: USE OF A PEPTIDE THAT INTERACTS WITH ALPHA V BETA3 INTEGRIN OF ENDOTHELIAL CELL



(57) Abstract: The present invention relates to the novel use of a peptide that interacts with the $\alpha v \beta 3$ integrin of endothelial cells. More particularly, the invention relates to a method for inhibiting endothelial cell adhesion, endothelial cell migration and/or angiogenesis, using a peptide consisting of at least 18 amino acids, comprising tyrosine-histidine (YH) or asparagine-histidine (NH), and at least three hydrophobic amino acids with bulky side chains; or equivalents thereof. Furthermore, the invention provides a method for treating or preventing angiogenesis-related diseases, using the peptide.



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**USE OF A PEPTIDE THAT INTERACTS WITH ALPHA V
BETA3 INTEGRIN OF ENDOTHELIAL CELL**

TECHNICAL FIELD

- 5 The present invention relates to a peptide having an angiogenesis-inhibitory effect, and more particularly, to the anti-angiogenic use of a peptide that interacts with the $\alpha v\beta 3$ integrin of endothelial cells.

BACKGROUND ART

- 10 Angiogenesis is defined as the formation of new capillary blood vessels from preexisting micro-vessels. Normal angiogenesis occurs during embryogenic development, tissue remodeling, organ growth, wound healing and female reproductive cycles (corpus luteum development) under tight physiological regulation (Folkman and Cotran, Int. Rev. Exp. Patho., 16:207-248, 1976).
- 15 Generally, angiogenesis involves the proteolysis of the blood vessel basement membrane by proteases, followed by the migration, proliferation and differentiation of endothelial cells to form tubules and eventually the regeneration of new blood vessels.

- Unregulated and abnormal angiogenesis may lead to various diseases.
- 20 Examples of angiogenesis-related diseases that occur in pathological conditions include various cancers(tumors); vascular diseases such as vascular malformation, arteriosclerosis, vascular adhesions, and edematous sclerosis; ocular diseases such as corneal graft neovascularization, neovascular glaucoma, diabetic retinopathy, angiogenic corneal disease, macular degeneration, pterygium, retinal degeneration,
- 25 retrolental fibroplasia and granular conjunctivitis; inflammatory diseases such as

rheumatoid arthritis, systemic Lupus erythematosus and thyroiditis; and dermatological diseases such as psoriasis, capillarectasia, pyogenic granuloma, seborrheic dermatitis and acne (USA Patent No. 5,994,292; Korean Patent Application Laid-Open No. 2001-66967; D'Amato R. J. *et al.*, *Ophtahlmol.*, 102:1261-1262, 1995; Arbiser J. L. *J. Am. Acad. Derm.*, 34(3):486-497, 1996; 5 O'Brien K. D. *et al.*, *Circulation*, 93(4):672-682, 1996; Hanahan D. *et al.*, *Cell*, 86:353-364, 1996).

Thus, studies on the mechanism of angiogenesis and the discovery of substances capable of inhibiting angiogenesis are of significant importance in the prevention and treatment of various diseases, including cancer. Current studies on the inhibition of angiogenesis are being performed on target genes by various strategies, including a strategy of administering a competitive substance to inhibit the action of VEGF and bFGF (basic fibroblast growth factor), which are known as potent inducers of angiogenesis, and a strategy of regulating the expression of integrin in vascular endothelial cells to inhibit the metastasis of the cancer cells. Regarding the relationship of angiogenesis with cancer, studies on the correlation between vascular absorption and angiogenesis induced by cancer cells and on proteins that induce angiogenesis are being performed but are still large incomplete. Studies on angiogenic inhibition are applicable to the diagnosis, treatment and/or prevention of a variety of angiogenesis-related diseases, and thus, there is a continued need for research and development regarding angiogenesis.

Meanwhile, β ig-h3 that is an extracellular matrix protein was first isolated by differential screening of a cDNA library made from a human lung adenocarcinoma cell line (A549) that had been treated with TGF- β 1 (Skonier J. *et al.*, 25

DNA Cell Biol., 11:511-522, 1992). The β ig-h3 protein consists of 683 amino acids and contains an amino-terminal secretory sequence and a carboxy-terminal RGD (Arg-Gly-Asp) motif serving as a ligand recognition site for several integrins (Skonier, J. *et al.*, DNA Cell Biol., 11:511, 1992). Also, the β ig-h3 protein contains
5 four homologous internal repeat domains (designated "fas-1 domains") which are homologous to similar motifs in the *Drosophila* fasciclin-I protein. Such fas-1 domains have highly conserved sequences found in the secretory and membrane proteins of many organisms, including mammals, insects, sea urchins, plants, yeast, and bacteria (Kawamoto T., *et al.*, Biochim. Biophys. Acta., 288-292, 1998). Each
10 of the fas-1 domains consists of 110-140 amino acids and comprises two highly conserved branches of about 10 amino acids (H1 and H2).

The β ig-h3 protein is known to have a fibrillar structure and to interact with several extracellular matrix proteins such as fibronectin and collagen (Kim J.-E., *et al.*, Invest. Ophthalmol. Vis. Sci., 43:656-661, 2002). Furthermore, the β ig-h3
15 protein has been reported to be involved in cell growth and differentiation, and wound healing and morphogenesis (Skonier J., *et al.*, DNA Cell Biol., 13:571-584, 1994; Dieudonne S. C., *et al.*, J. Cell. Biochem., 76:231-243, 1999; Kim J.-E., *et al.*, J. Cell. Biochem., 77:169-178, 2000; Rawe I. M., *et al.*, Invest. Ophthalmol. Vis. Sci., 38:893-900, 1997; and LeBaron R. G., *et al.*, J. Invest. Dermatol., 104:844-849,
20 1995). In addition, the β ig-h3 protein is known to mediate the adhesion of many different cell types, including corneal epithelial cells, chondrocytes and fibroblasts (LeBaron R. G., *et al.*, J. Invest. Dermatol., 104:844-849, 1995; Ohno S., *et al.*, Biochim. Biophys. Acta, 1451: 196-205, 1999; and Kim J.-E., *et al.*, J. Biol. Chem., 275:30907-30915, 2000). However, there is still no report indicating that the β ig-h3
25 protein is involved in angiogenesis.

DISCLOSURE OF THE INVENTION

Accordingly, the present inventors have performed extensive studies to determine whether the β ig-h3 protein is involved in angiogenesis, and consequently,
5 found that YH motif conserved in the fas-1 domains of the β ig-h3 protein shows an anti-angiogenic effect through the α v β 3 integrin of endothelial cells, thereby completing the present invention.

Therefore, an object of the present invention is to provide the novel use of a
10 peptide that interacts with the α v β 3 integrin of endothelial cells.

To achieve the above object, in one aspect, the present invention provides a method of inhibiting endothelial cell adhesion, endothelial cell migration and/or angiogenesis, comprising administering to a subject in need thereof an effective
15 amount of a peptide that interacts with the α v β 3 integrin of endothelial cells.

In another aspect, the present invention provides a method of treating or preventing angiogenesis-related diseases, comprising administering to a subject in need thereof an effective amount of a peptide that interacts with the α v β 3 integrin of endothelial cells.

20 In yet another aspect, the present invention provides a pharmaceutical composition for the inhibition of angiogenesis or for the treatment or prevention of angiogenesis-related diseases, comprising as active ingredient a peptide that interacts with the α v β 3 integrin of endothelial cells.

In still another aspect, the present invention provides the use of a peptide
25 interacting with the α v β 3 integrin of endothelial cells, for the preparation of a

pharmaceutical agent for the inhibition of endothelial cell adhesion, endothelial cell migration and/or angiogenesis.

In another further aspect, the present invention provides the use of a peptide interacting with the $\alpha v \beta 3$ integrin of endothelial cells, for the preparation of an agent
5 for the treatment or prevention of angiogenesis-related diseases.

As used herein, the term "YH motif" is defined as an amino acid sequence comprising tyrosine-histidine (Y-H) or asparagine-histidine (N-H) residues highly conserved in the fas-1 domains of a β ig-h3 protein, and flanking several hydrophobic
10 amino acid residues adjacent to the conserved residues(e.g., leucine and isoleucine) (Kim, J.-E. *et al.*, J. Biol. Chem., 277:46159-46465, 2002). The YH motif is also highly conserved in fas-1 domains derived from other proteins in addition to the β ig-h3 protein (see Fig. 1).

As used herein, the term "effective amount" is defined as an amount at
15 which the effect of inhibiting endothelial cell migration, endothelial cell adhesion and/or angiogenesis is shown.

As used herein, the term "subject" means animals, including mammals, particularly human beings. The subject may preferably be a patient who requires treatment.

20

Herein after, the present invention will be described in detail.

A peptide according to the present invention may consist of at least 18 amino acids, comprising tyrosine-histidine (Y-H) or asparagine-histidine (N-H) residues, and at least three hydrophobic amino acids with bulky side chains. The
25 hydrophobic amino acids with bulky side chains may be leucines (L) or isoleucines

(I). The inventive peptide comprises at least three of the hydrophobic amino acids, preferably four to six of them. The hydrophobic amino acids are preferably adjacent to the tyrosine-histidine (Y-H) or asparagines-histidine (N-H) residue. Concretely, the hydrophobic amino acids may be located at one side (N-terminal or
 5 C-terminal region) or both sides of the tyrosine-histidine (Y-H) or asparagines-histidine (N-H) residues.

Concretely, the inventive peptide may have an amino acid sequence comprising the YH motif which is conserved in the fas-1 domains. The inventive peptide may preferably consist of at least 18 amino acids, comprising the YH motif
 10 derived from each of the fas-1 domains of the β ig-3 protein. More preferably, the inventive peptide may comprises an amino acid sequence represented by (I, D, E or K)-(E, A or Q)-L-(L, R or A)-(N, D or S)-(A, L, K or I)-(L or Y)-(R, N, L or K)-(Y or N)-H-(M, I or G)-(V, L, Q or G)-(G, K, T or D)-(R, S, L or E)-(R, A, E or I)-(V, M, T or L)-(L, C or V)-(T, A, G or S). The amino acid abbreviations as described
 15 above have the following definitions: I, isoleucine; D, aspartate; E, glutamate; K, lysine; A, alanine; Q, glutamine; L, leucine; R, arginine; N, asparagine; S, serine; Y, tyrosine; H, histidine; M, methionine; G, glycine; V, valine; T, threonine; and C, cysteine. Most preferably, the inventive peptide may comprise an amino acid sequence selected from the group consisting of SEQ ID NO: 23 to SEQ ID NO: 26.
 20 The amino acid sequence of SEQ ID NO: 23 is derived from the first fas-1 domain of the β ig-h3 protein, and the amino acid sequence of SEQ ID NO: 24 from the second fas-1 domain of the β ig-h3 protein, the amino acid sequence of SEQ ID NO: 25 from the third fas-1 domain of the β ig-h3 protein, and the amino acid sequence of SEQ ID NO: 26 from the fourth fas-1 domain of the β ig-h3 protein.

It is understood that functional equivalents or salts of the peptide consisting of at least 18 amino acids, comprising tyrosine-histidine (Y-H) or asparagine-histidine (N-H) residues and at least three hydrophobic amino acids with bulky side chains, are within the scope of the inventive peptide. As used herein, the term
5 “functional equivalents” is defined as peptides where some amino acids in the inventive peptide are mutated to such a degree that they do not influence the physiological activity of the peptides. In other words, the peptides having the same or similar structure as the inventive peptide as well as amino acid sequences are within the scope of the present invention insofar as they show substantially the same
10 physiological activity as that of the inventive peptide. As used herein, the term “physiological activity” means the activity of inhibiting endothelial cell adhesion, endothelial cell migration and/or angiogenesis by the interacting with the $\alpha v \beta 3$ integrin of endothelial cells.

The mutation as described above includes the substitution, deletion and/or
15 addition of amino acids, and may be preferably the substitution of amino acids. An example of this mutation is the case where the hydrophobic amino acids with bulky side chains adjacent to the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) on the YH motif are substituted with other amino acids, and preferably serine. More preferably, the inventive peptide may also have either an amino acid sequence
20 selected from the group consisting of SEQ ID NO: 12 to SEQ ID NO: 14, or an amino acid sequence selected from the group consisting of SEQ ID NO: 18 to SEQ ID NO: 20, which comprises the amino acid sequence selected from SEQ ID NO: 12 to SEQ ID NO: 14.

The mutation may also be the case where the tyrosine-histidine (Y-H) or
25 asparagine-histidine (N-H) on the YH motif is substituted with two other amino

acids. Preferably, the tyrosine (Y) or asparagine (N) may be substituted with one selected from the group consisting of serine (S), histidine (H), phenylalanine (F) and threonine (T), and/or the histidine may be substituted with asparagine (N). More preferably, the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) may be substituted with amino acids selected from the group consisting of serine-histidine (S-H), histidine-histidine (H-H), phenylalanine-histidine (F-H), threonine-histidine (T-H) and tyrosine-asparagine (Y-N), which are conserved in the fas-1 domains of proteins known in the prior art (see Fig. 1).

Moreover, the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) may also be substituted with hydrophobic amino acids, and preferably alanine-alanine (A-A). In this case, the inventive peptide may have either an amino acid sequence of SEQ ID NO: 11, or an amino acid sequence of SEQ ID NO: 17, which comprises the amino acid sequence of SEQ ID NO: 11.

In addition, the mutation also includes the case where both the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) and the flanking hydrophobic amino acids with bulky side chains are substituted as described above. In this case, however, the hydrophobic amino acids with bulky side chains are preferably present at least three within the inventive peptide. Preferably, the inventive peptide may have either an amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16, or an amino acid sequence of SEQ ID NO: 21 or SEQ ID NO: 22, which comprises the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16.

Furthermore, the scope of the functional equivalents according to the present invention also encompasses peptide derivatives obtained by partially modifying the chemical structure of the inventive peptide while maintaining the backbone and physiological activity of the inventive peptide. Examples thereof

include structural modifications to modify the stability, storage, volatility and solubility of the inventive peptide.

The inventive peptide may be easily prepared by a chemical synthesis method known in the art (Creighton, *Proteins; Structures and Molecular Principles*,
5 W. H. Freeman and Co., NY, 1983). Typical methods includes but are not limited to liquid or solid state synthesis, fragment condensation, and F-MOC or T-BOC chemistry (*Chemical Approaches to the Synthesis of Peptides and Proteins*, Williams *et al.*, Eds., CRC Press, Boca Raton Florida, 1997; *A Practical Approach*, Atherton & Sheppard, Eds., IRL Press, Oxford, England, 1989).

10 Moreover, the inventive peptide may be prepared by a genetic engineering method. For this purpose, a DNA sequence encoding the inventive peptide is constructed according to a conventional method. The DNA sequence can be constructed by PCR-amplification with suitable primers. Alternately, the DNA sequence may also be synthesized by any standard method known in the art, for
15 example, using an automated DNA synthesis system (sold from Biosearch or Applied Biosystems). The constructed DNA sequence is inserted into a vector containing one or more expression control sequences (e.g., promoter and enhancer, etc.) which are operatively linked to the DNA sequence to control the expression of the DNA sequence. Host cells are then transfected with the resulting recombinant
20 expression vector. The transfected cells are incubated in a suitable medium and condition to express the DNA sequence, and a substantially pure peptide encoded by the DNA sequence is recovered. The peptide recovery may be performed by a method known in the art (e.g., chromatography). As used herein, the term "substantially pure peptide" means that the inventive peptide does not substantially
25 contain any peptides derived from host cells. For the genetic engineering method

for synthesizing the inventive peptide, reference may be made to the following publications: Maniatis *et al.*, Molecular Cloning; A laboratory Manual, Cold Spring Harbor laboratory, 1982; Sambrook *et al.*, supra; Gene Expression Technology, Method in Enzymology, Genetics and Molecular Biology, Method in Enzymology, Guthrie & Fink (eds.), Academic Press, San Diego, Calif, 1991; and Hitzeman *et al.*, J. Biol. Chem., 255:12073-12080, 1990.

Previously, the present inventors reported that the β ig-h3 protein has both $\alpha 3\beta 1$ integrin-interacting motif that induces the adhesion of epithelial cells (Kim, J.-E. *et al.*, J. Biol. Chem., 275:30907-30915, 2000), and $\alpha v\beta 5$ integrin-interacting motif that mediates the adhesion of fibroblasts (Kim, J.-E. *et al.*, J. Biol. Chem., 277:46159-46165, 2002). Then, in the present invention, whether the β ig-h3 protein and its fas-1 domains have endothelial cell adhesion activity was examined, and an integrin receptor that is involved in the adhesion of endothelial cells by the β ig-h3 protein was identified. Furthermore, to discover a motif within the β ig-h3 protein that interacts with the integrin receptor, deletion mutants of the β ig-h3 protein were prepared and their fragments, which are involved in cell adhesion, were examined (see Examples 1 to 4-1).

The results showed that the β ig-h3 protein and each of its fas-1 domains mediate the adhesion of endothelial cells at almost equal activity with each other (see Fig. 2), and that the $\alpha v\beta 3$ integrin is involved in the adhesion of endothelial cells by interacting with the β ig-h3 protein (see Figs. 3a to 3d). Furthermore, it was found that amino acids 548-614 (Δ H1H2(6)) of SEQ ID NO: 1, which corresponds to a fragment of the fas-1 domains, have activity related to the cell adhesion (see Fig. 4b).

Meanwhile, the present inventors previously reported that an YH motif that binds to the $\alpha v \beta 5$ integrin was present within a fragment corresponding to amino acids 548-614 of the β ig-h3 protein (Kim, L. E. *et al.*, J. Biol. Chem., 277:46159-46165, 2002). Thus, to confirm that the YH motif mediates endothelial cell adhesion by interacting with the $\alpha v \beta 3$ integrin, the present inventors constructed a fragment of 29 amino acids (amino acids 560-588 of SEQ ID NO: 1) comprising the YH motif, and relevant mutants whose tyrosine-histidine residues and flanking several isoleucines/leucines were substituted with alanine-alanine residues and serines, respectively, and examined their cell adhesion activity (see Example 4-2).

10 The results revealed that although various mutants of the YH motif have somewhat different activities, they all retain the activity of mediating the cell adhesion (see Fig. 4b).

Then, on the basis of the sequence of each fas-1 domain of the β ig-h3 protein, the present inventors synthesized four peptides (YH18 synthetic peptides represented by SEQ ID NOs: 23 to 26) consisting of 18 amino acids, which were designed to comprise the YH motif. The effect of the synthesized peptides on the adhesion of endothelial cells was tested (see Example 5).

20 The results showed that the YH18 synthetic peptides inhibit endothelial cell adhesion, which is mediated by the β ig-h3 protein, in a dose-dependent manner (see Fig. 5). This suggests that YH motif that is a conserved sequence in the fas-1 domains is responsible for endothelial cell adhesion to β ig-h3 protein through the $\alpha v \beta 3$ integrin.

Furthermore, in the present invention, whether or not the YH motif is involved in the migration of endothelial cells in addition to the adhesion of endothelial cells was tested (see Fig. 7). The results revealed that the YH18 synthetic peptide inhibits endothelial cell migration, which is promoted by the β ig-h3 protein, in a dose-dependent manner (see Figs. 7a and 7b).

Additionally, in the present invention, whether or not the YH motif that inhibits endothelial cell adhesion and migration shows an anti-angiogenic effect was tested (see Example 8). The results showed that angiogenesis is effectively inhibited by the YH18 synthetic peptide both *in vivo* and *in vitro* (see Figs. 8a and 8b).

10

The peptide according to the present invention has the following physiological activities:

First, the inventive peptide interacts with the $\alpha v \beta 3$ integrin of endothelial cells.

15 Second, it inhibits the adhesion and migration of endothelial cells.

Third, it inhibits angiogenesis both *in vitro* and *in vivo*.

Accordingly, the present invention provides a pharmaceutical composition for the inhibition of endothelial cell adhesion, endothelial cell migration and/or angiogenesis, comprising the inventive peptide as an active ingredient. Also, the present invention provides a pharmaceutical composition for the treatment or prevention of angiogenesis-related diseases, comprising the inventive peptide as an active ingredient.

20

The angiogenesis-related diseases that can be treated or prevented according to the present invention include various cancers(tumors); vascular diseases such as hemangioma, angiofibroma, vascular malformation, arteriosclerosis, vascular adhesions, and edematous sclerosis; ocular diseases such as corneal graft
5 neovascularization, neovascular glaucoma, diabetic retinopathy, angiogenic corneal disease, macular degeneration, pterygium, retinal degeneration, retrolental fibroplasia and granular conjunctivitis; inflammatory diseases such as rheumatoid arthritis, systemic Lupus erythematosus and thyroiditis; and dermatological diseases, such as psoriasis, capillarectasia, pyogenic granuloma, seborrheic dermatitis and acne (USA
10 Patent No. 5,994,292; Korean Patent Application Laid-Open No. 2001-66967; D'Amato R. J. *et al.*, *Ophtahlmol.*, 102:1261-1262, 1995; Arbiser J. L. *J. Am. Acad. Derm.*, 34(3):486-497, 1996; O'Brien K. D. *et al.*, *Circulation*, 93(4):672-682, 1996; Hanahan D. *et al.*, *Cell*, 86:353-364, 1996). More preferred examples include cancers, arthritis, psoriasis, diabetic eye diseases, arteriosclerosis, and inflammation.

15

The pharmaceutical composition comprising the inventive peptide as an active ingredient may further comprise a pharmaceutically acceptable carrier, for example, a carrier for oral or parenteral administration. Examples of the carrier for oral administration include lactose, starch, cellulose derivatives, magnesium stearate,
20 and stearic acid. For oral administration, the inventive peptide may be mixed with an excipient and used in various forms, including enteric tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups and wafers. Also, examples of the carrier for parenteral administration includes water, suitable oils, saline solution, aqueous glucose and glycol, and the inventive composition may further comprise
25 stabilizers and conservatives. Suitable examples of the stabilizers include

antioxidants, such as sodium hydrogensulfite, sodium bisulfite and ascorbic acid. Suitable examples of the preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol. For other pharmaceutically acceptable carriers, reference may be made to the following literature: Remington's Pharmaceutical Sciences, 19th ed., Mack Publishing Company, Easton, PA, 1995.

The pharmaceutical composition according to the present invention may be formulated in various forms for oral or parenteral administration. The formulations for parenteral administration are typically injection formulations, and preferably isotonic aqueous solution or suspension. The injection formulations may be prepared using suitable dispersing or wetting agents, and suspending agents, according to any technique known in the art. For example, the components may be formulated for injection by dissolving them in a saline or buffer solution. Examples of the formulations for oral administration include tablets and capsules, and these formulations may comprise diluents (e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose, and/or glycin) and lubricants (e.g., silica, talc, stearic acid and magnesium or calcium salts thereof, and/or polyethylene glycol), in addition to the active ingredient. The tablets may comprise binders such as magnesium aluminum silicate, starch paste, gelatin, tragacanth, methyl cellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and occasionally, it may further comprise disintegrants such as starch, agar, alginate or a sodium salt thereof, absorbing agents, coloring agents, flavoring agents and/or sweetening agents on a case by case basis. These formulations may be prepared by a conventional method such as mixing, granulation or coating.

The pharmaceutical composition according to the present invention may additionally comprise aids such as preservatives, wettable powders, emulsifiers, salts

for the regulation of osmotic pressure, and/or buffers, and other therapeutically useful materials. The pharmaceutical composition may be formulated according to a conventional method.

5 The total amount of the inventive peptide as an active ingredient in the inventive pharmaceutical composition can be administered to a subject as a single dose over a relatively short period of time, or can be administered using a fractionated treatment protocol where multiple doses are administered over a prolonged period of time. Although the content of the inventive peptide in the
10 inventive pharmaceutical composition can vary depending on the severity of diseases, the inventive peptide can be generally administered several times a day at a dose of 10 µg-10 mg. However, the dose of the inventive peptide depends on many factors, including the age, weight, general health, sex, disease severity, diet and excretion of a subject, as well as the route of administration and the number of treatments to be
15 administered. In view of these factors, any person skilled in the art would adjust the particular dose so as to obtain an effective dose for inhibiting angiogenesis, or for treating or preventing angiogenesis-related diseases. The pharmaceutical composition according to the present invention is not specifically limited in its formulation, administration route and administration mode insofar as it shows the
20 effects of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the comparison between amino acid sequences which contain
25 the tyrosine-histidine residues that are highly conserved in fas-1 domains derived

from various proteins, and the conserved leucine/isoleucine residues adjacent to the tyrosin-histidine residues.

Fig. 2 is a schematic diagram (A) showing recombinant proteins containing each fas-1 domain of a β ig-h3 protein, and a graphic diagram (B) showing the adhesion of HUVECs to the plate coated with β ig-h3 or each of its fas-1 domains, in terms of absorbance.

BSA: bovine serum albumin as control

Wild-type: recombinant β ig-h3 His- β -b protein containing all of four fas-1 domains.

- 1.0 D-I: first fas-1 domain
- D-II: second fas-1 domain
- D-III: third fas-1 domain
- D-IV: fourth fas-1 domain

Fig. 3a is a graphic diagram showing the inhibition of HUVECs adhesion to the β ig-h3 coated on the plate by function-blocking antibodies against various integrins.

- BSA: plate coated with BSA
- None: no treatment
- α 3: treated with P1B5 (antibody to α 3)
- 20 α 5: treated with P1D6 (antibody to α 5)
- α v: treated with P3G8 (antibody to α v)
- β 1: treated with 6S6 (antibody to β 1)
- β 3: treated with B3A (antibody to β 3)
- α v β 3: treated with LM609 (antibody to α v β 3)
- 25 α v β 5: treated with P1F6 (antibody to α v β 5)

Fig. 3b is a graphic diagram showing the inhibition of HUVECs adhesion to each of fas-1 domains coated on the plate by a function-blocking antibody against $\alpha\beta 3$ or $\alpha\beta 5$ integrin.

BSA: plate coated with BSA

5 D-I: first fas-1 domain of β ig-h3

D-II: second fas-1 domain of β ig-h3

D-III: third fas-1 domain of β ig-h3

D-IV: fourth fas-1 domain of β ig-h3

10 Fig. 3c shows the results of FACS analysis for the expression of integrin on the HUVECs surface using a function-blocking antibody against $\alpha\beta 3$ or $\alpha\beta 5$ integrin.

Fig. 3d shows the results of dose-dependent Western-immunoblotting analysis for the binding ability of biotin- β ig-h3 to a HUVECs cell membrane (A), and for the inhibition of biotin β ig-h3 binding to the HUVECs cell membrane by a
15 function-blocking antibody against $\alpha\beta 3$ or $\alpha\beta 5$ integrin (B), in which the concentration-dependent Western-immunoblotting analysis is performed to identify a receptor for β ig-h3 that is involved in endothelial cell adhesion. β -tubulin is an internal control for equal protein loading.

20 Fig. 4a is a schematic diagram showing various deletion mutants of the fourth fas-1 domain (D-IV) of β ig-h3.

Fig. 4b schematically shows various substitution mutants of an YH motif conserved in the fourth fas-1 domain, and graphically shows test results for the HUVECs adhesion activity of the mutants.

Black blocks: substituted amino acids.

Fig. 5 shows the amino acid sequences of YH18 synthetic peptides derived from each fas-1 domain of β ig-h3, and graphically shows the dose-dependent inhibition of HUVECs adhesion to β ig-h3 by the peptides.

Fig. 6a is a graphic diagram showing the adhesion of HEK293 cells
5 transfected with a β 3 integrin expression vector to β ig-h3.

pc/293: HEK293 cells transfected with pcDNA3 vector

β 3/293: HEK293 cells transfected with β 3 integrin expression vector

Fig. 6b is a graphic diagram showing the inhibition of β 3/293 cell adhesion
to β ig-h3 coated on the plate by a function-blocking antibody against α v β 3 or α v β 5
10 integrin.

BSA: plate coated with BSA

IgG: treated with mouse IgG

α v β 3: treated with LM609 (antibody to α v β 3)

α v β 5: treated with P1F6 (antibody to α v β 5)

Fig. 6c is a graphic diagram showing the dose-dependent inhibition of
15 β 3/293 cell adhesion to β ig-h3 coated on the plate by YH18 synthetic peptides.

Fig. 7a is a photograph (A) and a graphic diagram (B), which show the
inhibition of HUVECs migration toward β ig-h3 coated on the plate by a function-
blocking antibody against α v β 3 or α v β 5 integrin.

20 BSA: plate coated with BSA

IgG: treated with mouse IgG

α v β 3: treated with LM609 (antibody to α v β 3)

α v β 5: treated with P1F6 (antibody to α v β 5)

Fig. 7b is a photograph (A) and a graphic diagram (B), which show the inhibition of HUVECs migration toward β ig-h3 coated on the plate by an YH18 synthetic peptide.

BSA: plated with BSA

5 control: treated with 5% DMSO

YH18-con.: treated with YH18-con. peptide (control peptide)

YH18-500 μ M: treated with 500 μ M YH18 synthetic peptide

YH18-1mM: treated with 1 mM YH18 synthetic peptide

10 Fig. 8a is a photograph (A) showing the inhibition of HUVECs tube formation by an YH18 synthetic peptide, and a graphic diagram (B) showing the result of measurement for the number of tube branches formed.

Control: treated with 5% DMSO

YH18-con.: treated with YH18-con. peptide (control peptide)

YH18-500 μ M: treated with 500 μ M YH18 synthetic peptide

15 YH18-1 mM: treated with 1 mM YH18 synthetic peptide

Fig. 8b is a photograph (A) showing the inhibition of angiogenesis by the YH18 synthetic peptide, a photograph (B) showing a section stained with H&E, and a graphic diagram (C) showing the result of measurement for the number of blood vessels.

20

BEST MODE FOR CARRYING OUT THE INVENTION

Hereinafter, the present invention will be described in detail by examples. It will however be obvious to a person skilled in the art that the present invention is not limited to or by the examples.

25

Example 1: Expression and purification of β ig-h3 protein and its fas-1 domains

1-1: Construction of expression vector

Previously, the present inventors reported recombinant proteins comprising
5 β ig-h3 and each of its fas-1 domains (Kim, J.-E. *et al.*, J. Biol. Chem., 275:30907-30915, 2000; and Korean Patent Registration No. 10-0382042). Thus, β ig-h3 and its fas-1 domains were prepared in the same manner as described in the report.

Concretely, a recombinant human β ig-h3 protein (hereinafter, referred to as “ β ig-h3 His- β -b”) that expresses all of four fas-1 domains was prepared using a
10 pHis- β -b vector. The pHis- β -b vector had been prepared by inserting an *Asp*718-*Bgl*III fragment (obtained by partially deleting the amino terminal region of β ig-h3 cDNA) into the *EcoRV/EcoRI* sites of pET-29 β . Also, to express recombinant proteins containing each fas-1 domain of human β ig-h3, the present inventors PCR-amplified four cDNA fragments of β ig-h3, which include the first fas-1 domain (β ig-h3 D-I), the second fas-1 domain (β ig-h3 D-II), the third fas-1 domain (β ig-h3 D-III)
15 or the fourth fas-1 domain (β ig-h3 D-IV), respectively (see A of Fig. 2).

Then, each of the PCR products was cloned into the *EcoRV/XhoI* sites of a pET-29b(+) vector (Novagen; Madison, WI). The constructed expression vectors were named “p β ig-h3 D-I”, “p β ig-h3 D-II”, “p β ig-h3 D-III” and “p β ig-h3 D-IV”, respectively. The amino acid sequences of the four fas-1 domains of β ig-h3 are represented by SEQ ID NO: 2 to SEQ ID NO: 5,
20 respectively.

1-2: Transformation of E. coli and purification of recombinant protein

E. coli BL21 (DE3) cells were transformed with each of the expression vectors constructed in Example 1-1. The transformed *E. coli* cells were incubated in LB medium containing 50 µg/ml kanamycin. To induce the expression of each recombinant protein, when the absorbance of the culture reached 0.5-0.6 at a 595 nm, the culture was added with 1 mM IPTG (isopropyl-β-D-(-)-thiogalactopyranoside) and further incubated at 37 °C for three hours. Next, purification of the expressed proteins was performed according to the method described by Kim, J.-E. *et al.*, J. Cell. Biochem., 77:169-187, 2000. For this purpose, the cells were collected by centrifugation and resuspended in buffer (50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM PMSF, 0.5 mM DTT). The cell suspension was disrupted by sonication. The proteins expressed in the form of an inclusion body were dissolved in an 8M urea-denaturation buffer, and the denaturated proteins were purified with a Ni-NTA resin (Qiagen). The recombinant proteins were eluted in 200 mM imidazole solution, and purified by dialysis against 20 mM Tris-HCl buffer containing 50 mM sodium chloride in a stepwise manner from high to low urea concentration. The expressed and purified proteins were analyzed by SDS-PAGE (data not shown). Unlike the recombinant protein βig-h3 His-β-b containing all of the four fas-1 domains, the recombinant proteins containing each of the four fas-1 domains were synthesized in a water-soluble form and thus did not require a modification step. Also, they could be easily obtained in large amounts.

Meanwhile, the *E. coli* transformed with the expression vectors pHis-β-b, pβig-h3 D-II and pβig-h3 D-IV were termed "*E. coli* BL21/His-β-b", "*E. coli* BL21/Hisβ-g" and "*E. coli* BL21/Hisβ-e", respectively, and deposited in the Korean Collection for Type Cultures (KCTC), Korean Research Institute of Bioscience and

Biotechnology, under accession numbers KCTC 18008P, KCTC 18010P and KCTC 18009P, respectively, on April 25, 2000.

Example 2: Test of endothelial cell adhesion activity of β ig-h3 and its fas-1

5 domains

Cell adhesion assay was performed according to the method described by Kim, J.-E. *et al.*, J. Biol. Chem., 277:46159-46165, 2002. For this purpose, 10 μ g/ml of each of the recombinant proteins (β ig-h3 His- β -b, β ig-h3 D-I, β ig-h3 D-II, β ig-h3 D-III and β ig-h3 D-IV) prepared in Example 1 was placed into a flat-bottomed 96-well ELISA (enzyme-linked immunosorbent assay) plate (Costar, Corning Inc., NY) and then incubated overnight at 4 °C, to coat the surface of the plate. As a control, 2% BSA was coated on the plate. Then, the plate was treated with PBS (phosphate-buffered saline) containing 2% BSA, and blocked at room temperature for one hour.

15 Meanwhile, HUVECs (human umbilical vein endothelial cells; Clonetics, San Diego, CA) were incubated in EGM medium (Clonetics, San Diego, CA) containing 2% FBS (Fetal Bovine Serum) under a condition of 37 °C and 5% CO₂. The incubated cells were suspended in medium at a density of 3×10^5 cells/ml, and 0.1 ml of the cell suspension was added to each well of the plate. Next, the cells were incubated at 37 °C for 30 minutes and washed one time with PBS buffer to remove cells which had not been attached to the plate. Attached cells were added with 50 mM citrate buffer (pH 5.0) containing 3.75 mM p-nitrophenyl-N-acetyl β -D-glycosaminide and 0.25% Triton X-100, and incubated at 37 °C for one hour. Thereafter, 50 mM glycine buffer (pH 10.4) containing 5 mM EDTA was added to
25 block the activity of the enzyme. The absorbance was measured at a 405-nm in a

Bio-Rad model 550 microplate reader. Here, the higher the number of cells adhered to the plate, the higher the absorbance.

The results showed that, as shown in B of Fig. 2, β ig-h3 mediated the adhesion of endothelial cells, and each of the fas-1 domains of β ig-h3 also mediated the adhesion of endothelial cells with an almost equal activity to that of β ig-h3.

Example 3: Identification of integrins that are involved in adhesion of endothelial cells to β ig-h3

3-1: Test 1 for identification of integrin receptors

To identify integrins that are involved in the adhesion of endothelial cells to β ig-h3, the present inventors have performed a cell adhesion inhibition assay using various antibodies that specifically blocks the function of integrins.

For this purpose, 5 μ g/ml of monoclonal antibodies specific to different types of integrin (Chemicon, International Inc, Temecula, CA) was preincubated at 37 °C for 30 minutes with HUVECs in 0.1 ml of the cell suspension (3×10^5 cells/ml). The following antibodies were used in this test: P1B5 (antibody to $\alpha 3$), P1D6 (antibody to $\alpha 5$), P3G8 (antibody to αv), 6S6 (antibody to $\beta 1$), B3A (antibody to $\beta 3$), LM609 (antibody to $\alpha v \beta 3$) and P1F6 (antibody to $\alpha v \beta 5$). A culture which had not been preincubated with the antibody was used as a control. Then, the incubated cells were transferred onto plates precoated with the recombinant protein β ig-h3 His- β -b and incubated at 37 °C for 30 minutes. The attached cells were then quantified in the same manner as in Example 2. The results showed that, as shown in Fig. 3a, the adhesion of endothelial cells to β ig-h3 was inhibited specifically by the antibodies to $\alpha v \beta 3$ integrin and $\beta 3$ integrin, but it was not inhibited by the antibodies to other integrins, including $\alpha 3$ and $\alpha 5$.

3-2: Test 2 for identification of integrin receptors

In Example 2 above, it was confirmed that β ig-h3 and also its fas-1 domains mediate the adhesion of endothelial cells. Thus, in order to identify an integrin receptor for each of the fas-1 domains of β ig-h3, the present inventors coated a plate surface with each of the fas-1 domains of β ig-h3 and performed a cell adhesion inhibition assay.

The results showed that, as shown in Fig. 3b, the adhesion of endothelial cells to each of the fas-1 domains was inhibited specifically by the antibody to α v β 3, but it is not inhibited by the antibody to α v β 5.

3-3: Confirmation of integrins that are expressed on surface of endothelial cells

To confirm that HUVECs express both α v β 3 and α v β 5 integrin on their surface, the present inventors performed an FACS analysis using monoclonal antibodies specific to the two integrins.

For this purpose, a plate in which HUVECs had been grown to confluence was treated with PBS buffer containing 0.25% trypsin and 0.05% EDTA to detach the cells from the plate surface. The cells were washed two times with PBS buffer and resuspended in PBS buffer. The cell suspension was added with an anti- α v β 3 integrin antibody (LM609; Chemicon, International Inc, Temecula, CA) or an anti- α v β 5 integrin antibody (PIF6; Chemicon, International Inc, Temecula, CA) and incubated at 4 °C for one hour. The cells were then further incubated for one hour at 4 °C with 10 μ g/ml of a FITC-conjugated secondary goat antimouse IgG antibody (Santa Cruz Biotechnology, Inc., CA). The resulting cells were analyzed at 488 nm

on the flow cytometer FACSCalibur system (Becton Dickinson, San Jose, CA) equipped with a 5-watt laser. A control was incubated with a secondary antibody alone.

The results showed that, as shown in Fig. 3c, HUVECs expressed both the $\alpha v \beta 3$ integrin and the $\alpha v \beta 5$ integrin. However, the expression level of the $\alpha v \beta 5$ integrin was far less than that of the $\alpha v \beta 3$ integrin.

3-4: Test 3 for identification of integrin receptors

To confirm that HUVECs adhesion that is mediated by β ig-h3 depends on $\alpha v \beta 3$ integrin, the present inventors tested the binding affinity of β ig-h3 in the presence of an antibody that specifically blocks the function of the integrin. This binding assay was performed according to the method described by Maile, L. A., *et al.*, J. Biol. Chem., 275:23745-23750, 2002.

First, HUVECs were suspended in medium at a density of 1×10^5 cells/ml. 1 ml of the cell suspension was preincubated with anti- $\alpha v \beta 3$ antibody (LM609) or anti- $\alpha v \beta 5$ antibody (P1F6) at 37 °C for 30 minutes. Thereafter, the preincubated cells were incubated with biotinylated β ig-h3 (hereinafter, referred to as "biotin- β ig-h3") in a serum-free medium containing 0.1% BSA at 4 °C for 5 hours. The biotin- β ig-h3 was added at concentrations of 1×10^{-10} , 1×10^{-9} and 5×10^{-9} μ M, respectively. Then, the cells were washed three times with PBS buffer (pH 7.4), and dissolved at 4 °C in ice-cold buffer A (10mM Tris-Cl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.5% SDS, 0.02% sodium azide, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride). The cell lysates were clarified by centrifugation at 13,000 rpm for 30 minutes at 4 °C. Equal amounts of protein were

then separated by SDS-PAGE, 8% gel. The amount of biotin- β ig-h3 was determined by Western immuno-blotting.

To visualize the biotin- β ig-h3, the membranes were incubated with HRP (hoseradish peroxidase; Amersham Biosciences)-conjugated streptavidin. Next,
5 Binding of the peroxidase-labeled antibody was visualized using ELC (enhanced chemiluminescence; Amersham Biosciences). As an internal control, a β -tubulin protein was subjected to Western blotting to verify equal protein loading.

The results showed that β ig-h3 was bound to HUVECs surface in a dose-dependent manner (see A of Fig. 3d), and its binding was specifically inhibited only
10 by the antibody to α v β 3 integrin (see B of Fig. 3d).

Such results suggest that each of the fas-1 domains of β ig-h3 contains a motif that mediates the adhesion of endothelial cells through the ' α v β 3 integrin', but not the α v β 5 integrin.

15

Example 4: Identification of β ig-h3 motif interacting with α v β 3 on endothelial cell adhesion

4-1: Identification of α v β 3 integrin-interacting motifs using deletion mutants of fas-1 domains

20 To identify motifs that interact with the α v β 3 integrin when β ig-h3 or its fas-1 domains induce the adhesion of endothelial cells by interacting with the α v β 3 intergrin, the present inventors prepared deletion mutants of the fas-1 domains and tested their cell adhesion activity.

For this purpose, deletion mutants of the fas-1 domains were prepared
25 according to the method described by Kim, J.-E. *et al.*, J. Biol. Chem., 275:30907-

30915, 2000. Concretely, in the present invention, there were prepared several deletion mutant fragments which lack a H1 or H2 peptide, which is highly conserved in the fas-1 domains, and/or an EPDIM motif, which is involved in the adhesion of corneal epithelial cells by interacting with the $\alpha 3\beta 1$ integrin (see Fig. 4a).

- 5 Information of the deletion mutant fragments is given in Table 1 below. The amino acid region of each of the deletion mutant fragments is based on the amino acid sequence of β ig-h3, which is represented by SEQ ID NO: 1.

Table 1: Deletion mutants of fas-1 domains

10

No.	Amino acid region of deletion mutant fragment	Designation	Characteristic	SEQ ID NO
1	548-632	Δ H1	5'-terminal fragment containing H1 peptide was deleted.	6
2	502-620	Δ H2	H2 peptide was deleted.	7
3	502-614	Δ H2(6)	EPDIM and H2 peptide were deleted.	8
4	548-620	Δ H1H2	5'-terminal fragment containing H1 peptide, and H2 peptide, were deleted.	9
5	548-614	Δ H1H2(6)	5'-terminal fragment containing H1 peptide, H2 peptide and EPDIM, were deleted.	10

Each of the deletion mutants was generated by PCR using a cDNA template encoding the fourth fas-1 domain (SEQ ID NO: 5) (Kim, J.-E. *et al.*, J. Biol. Chem., 275:30907-30915, 2000). Each of the PCR-amplified DNA fragments was cloned into the *EcoRV/XhoI* sites of the pET-29b(+) vector (Novagen; Madison, WI). The mutations were confirmed by sequence analysis, and the deletion mutants were expressed and purified according to a prior method (Kim, J.-E., *et al.*, J. Cell. Biochem., 77:169-178, 2000). Thereafter, the cell adhesion activity of each of the deletion mutants was tested in the same manner as in Example 2.

The results showed that $\Delta H1H2(6)$, which is the smallest deletion mutant fragment, still retains endothelial cell adhesion activity (data not shown). This suggests that $\alpha v\beta 3$ integrin-interacting motif is present within a fragment corresponding to amino acids 548-614 of SEQ ID NO: 1.

4-2: Identification of $\alpha v\beta 3$ integrin-interacting motif using YH motif

mutants

Previously, the present inventors reported that an YH motif, which contains tyrosine-histidine residues highly conserved in the fragment corresponding to amino acids 548-614 of SEQ ID NO: 1, and several leucine and isoleucine residues adjacent to the tyrosine-histidine residues, binds to the $\alpha v\beta 5$ integrin (Kim, J.-E., *et al.*, J. Biol. Chem., 277:46159-46165, 2002). Thus, the present inventors suspected that the YH motif may also interact with the $\alpha v\beta 3$ integrin to mediate endothelial cell adhesion. For this reason, in the present invention, the tyrosine-histidine residues conserved in the fas-1 domains, and/or the leucine and isoleucine residues adjacent to the tyrosine-histidine residues, were substituted in various combinations by alanine or serine (Kim, J.-E., *et al.*, J. Biol. Chem. 277:46159-46165, 2002)(see Fig.

4b). The amino acid sequences of the prepared YH motif mutants are shown in SEQ ID NO: 17 to SEQ ID NO: 22. The mutations were confirmed by sequence analysis. Then, the substitution mutants were expressed and purified according to a known method (Kim, J.-E., *et al.*, J. Cell. Biochem., 77:169-178, 2000). The substitution mutants were tested for their cell adhesion activity according to the same manner as in Example 2.

The results shown in Fig. 4b confirmed that, in a case of D-IV-AA where the tyrosine-histidine residues highly conserved in the fas-1 domains were substituted with alanine-alanine residues that are hydrophobic amino acids, and in cases of D-IV-L and D-IV-R where the leucine and isoleucine residues adjacent to either side (N- or C-terminal region) of the tyrosine-histidine residues were substituted with a hydrophilic amino acid, serine, cell adhesion activity was equal to those of the fas-1 domain (D-IV) and its fragment (H1H2(6)). Meanwhile, in a case of D-IV-LYHR where all of the isoleucine and leucine residues present in both side of the tyrosin-histidine residues were substituted with serine, cell adhesion activity was reduced to half that of the fas-1 domain. Also, in cases of D-IV-LAA and D-IV-AAR where both the tyrosine-histidine residues and the isoleucine and leucine residues adjacent to either side of the tyrosine-histidine residues were substituted with the alanine-alanine residues and the serin residue, respectively, cell adhesion activity was somewhat lower than those of D-IV-LAA and D-IV-AAR. However, all the substitution mutants showed a higher cell adhesion activity than that of a control. Theses results suggest that not only the tyrosine-histidine residues but also the hydrophobic amino acids with bulky side chains, which are adjacent to both sides of the tyrosine-histidine residues, are required for the adhesion of endothelial cells.

Example 5: Assay of inhibition of endothelial cell adhesion by YH18 synthetic peptide

To further confirm that the YH motif within β ig-h3 is involved in endothelial cell adhesion, the present inventors performed cell adhesion assay using YH18 synthetic peptides (Kim, J.-E., *et al.*, J. Biol. Chem., 277:46159-46165, 2002). The YH18 synthetic peptides, which are derived from the YH motif conserved in all the fas-1 domains of β ig-h3, are peptides of 18 amino acids containing tyrosine (or asparagine)-histidine residues and flanking several leucine and isoleucine residues.

Concretely, in this Example, the following YH18 synthetic peptides were used: a YH18 synthetic peptide of SEQ ID NO: 23 derived from the first fas-1 domain of β ig-h3 (hereinafter, referred to as "D-I YH18"), a YH18 synthetic peptide of SEQ ID NO: 24 derived from the second fas-1 domain (hereinafter, referred to as "D-II YH18"), a YH18 synthetic peptide of SEQ ID NO: 25 derived from the third fas-1 domain (hereinafter, referred to as "D-III YH18"), and a YH18 synthetic peptide of SEQ ID NO: 26 derived from the fourth fas-1 domain (hereinafter, referred to as "D-IV YH18") (see Fig. 5). As a control peptide, an YH18-con. peptide of SEQ ID NO: 27 was used. All the above peptides had been synthesized by AnyGen Co. Ltd, Kwangju, Korea. Thereafter, the present inventors tested whether the YH18 synthetic peptides have the ability to inhibit endothelial cell adhesion in the wells coated with β ig-h3.

For this purpose, HUVECs were suspended in medium at a density of 3×10^5 cells/ml, and each of the YH18 synthetic peptides and the YH-con. peptide was added to 0.1 ml of the cell suspension at concentrations of 100 μ M, 300 μ M, 500 μ M and 1,000 μ M, respectively. The peptide-containing cell suspensions were

preincubated at 37 °C for 30 minutes. The preincubated cells were added to each well of a 96-well plate precoated with a recombinant β ig-h3 His- β -b protein. Then, the cells were tested in the same manner as in Example 2.

The results showed that, as shown in Fig. 5, the adhesion of endothelial cells, which is mediated by β ig-h3, is inhibited by the YH18 synthetic peptides derived from the fas-1 domains of β ig-h3, in a dose-dependent manner. This suggests that the YH motifs are involved in endothelial cell adhesion to β ig-h3 through the α v β 3 integrin.

10 Example 6: Reconfirmation of functional receptor α v β 3 of endothelial cells for β ig-h3

To reconfirm that the α v β 3 integrin mediates the adhesion of endothelial cells to β ig-h3, a cell adhesion assay was performed using HEK293 cells which had been stably transfected with a human β 3 integrin expression vector.

15 6-1: Cell adhesion assay

To construct a human β 3 integrin expression vector, RT-PCR was performed using a human placenta poly(A)⁺ RNA as a template, thereby generating a 2.4-kb β 3 cDNA (Chandrika, S. K. *et al.*, J. Biol. Chem., 272:16390-16397, 1997). The amplified β 3 cDNA was digested with *HindIII/XbaI*, and then cloned into the pcDNA3 vector (Invitrogen). The resulting vector was named "β3/pcDNA3". Then, 1 μg of the β3/pcDNA3 vector was introduced into HEK293 cells (ATCC, catalog No. CRL 1573) using lipofectamin (Gibco). A control was introduced with a pcDNA3 vector containing no human β 3 cDNA. Since all the vectors contain a G418 selection marker, the stable transfected cells were screened using 1 mg/ml of G418. The cell transfected with the β3/pcDNA3 vector was

20
25

named " $\beta 3/293$ ", and the cell transfected with the pcDNA3 vector (control) was named " $pc/293$ ". Each of the screened transfectants was incubated in DMEM (Dulbecco's Modified Eagle Medium) containing 10% FBS, streptomycin and penicillin. Then, cell adhesion assay was performed in the same manner as in

5 Example 2.

The results showed that, as shown in Fig. 6a, the $\beta 3/293$ cells were strongly adhered to β ig-h3, whereas the $pc/293$ cells were not adhered to β ig-h3, in a similar manner to the plate coated with BSA. This is because the $pc/293$ cells do not synthesize $\beta 3$ while producing only αv by themselves and thus do not adhered to β ig-

10 h3, but the $\beta 3/293$ cells can express $\beta 3$ to produce the $\alpha v\beta 3$ integrin.

6-2: Assay of inhibition of endothelial cell adhesion using integrin function-blocking antibodies

In the present invention, to further confirm that β ig-h3 mediates endothelial cell adhesion through the $\alpha v\beta 3$ integrin, cell adhesion assay using antibodies that

15 block the function of integrins was performed in the same manner as in Example 3-1.

The results showed that, as shown in Fig. 6b, the adhesion of $\beta 3/293$ cells to β ig-h3 was inhibited specifically by a function-blocking antibody against the $\alpha v\beta 3$ integrin, whereas the adhesion is not inhibited by an antibody to $\alpha v\beta 5$.

20

6-3: Assay of inhibition of endothelial cell adhesion using YH18 synthetic peptide

In Example 5, the present inventors confirmed that the YH18 synthetic peptides inhibit endothelial cell adhesion to β ig-h3 through the $\alpha v\beta 3$ integrin. Then,

25 the present inventors tested whether the YH18 synthetic peptide also inhibits the

adhesion of $\beta 3/293$ cells to β ig-h3. The test was performed in the same manner as in Example 5.

The results showed that, as shown in Fig. 6c, the YH18 synthetic peptides derived from each of the fas-1 domains inhibit the adhesion of $\beta 3/293$ to β ig-h3 in a
5 dose-dependent manner.

From the above results, it was reconfirmed that the YH motif in each fas-1 domain of β ig-h3 mediates endothelial cell adhesion through $\alpha v\beta 3$, and a peptide containing the YH motif inhibits the endothelial cell adhesion by interacting with the
10 $\alpha v\beta 3$ integrin.

Example 7: Assay of inhibition of endothelial cell migration by YH18 synthetic peptide

7-1: Assay of inhibition of endothelial cell migration using integrin
15 function-blocking antibody

Firstly, to examine whether β ig-h3 is involved in the migration of endothelial cells, the present inventors performed cell migration assay. The cell migration assay was performed in a transwell plate (8 μ m pore size, Costar, Cambridge, MA). The undersurface of the membrane was coated with the
20 recombinant β ig-h3 His- β -b protein (10 μ g/ml) prepared in Example 1 at 4 °C, and then, blocked for 1 hour at room temperature with PBS buffer containing 2% BSA. Meanwhile, HUVECs were added with an anti- $\alpha v\beta 3$ antibody (LM609) or an anti- $\alpha v\beta 5$ antibody (P1F6) and preincubated at 37 °C for 30 minutes. A control was added with mouse IgG. Then, the HUVECs preincubated with the antibody were
25 suspended in medium at a density of 3×10^5 cells/ml, and 0.1 ml of the cell

suspension was added to the upper compartment of the filter. The cells were allowed to migrate at 37 °C for 6-8 hours.

Migration was terminated by removing the cells from the upper compartment of the filter with a cotton swab. The filters were fixed with 8% glutaraldehyde and stained with crystal violet. The extent of cell migration was determined by light microscope, and within each well, cell counting was done in nine randomly selected fields HPF (Microscopic high power fields, x200).

The results showed that the migration of HUVECs was enhanced in those transwells whose undersurface was coated with β ig-h3 (data not shown) and this effect was inhibited specifically by an antibody to the α v β 3 integrin but not an antibody to the α v β 5 integrin (see Fig. 7a).

7-2: Assay of inhibition of endothelial cell migration by YH18 synthetic peptide

Then, the present inventors examined whether the migration of endothelial cells is inhibited by the YH18 synthetic peptide. For this purpose, a test was performed in the same manner as in Example 7-1 except that 500 μ M or 1 mM of an YH18 synthetic peptide represented by SEQ ID NO: 26 instead of the integrin function-blocking antibody was added together in adding the HUVECs suspension to the upper compartment of the membrane. A control to the peptide treatment was treated with 5% DMSO (solvent), and a control to the YH synthetic peptide added with an YH18-con. peptide represented by SEQ ID NO: 27.

The test results showed that, as shown in Fig. 7b, the YH18 synthetic peptide also inhibited the migration of endothelial cells toward β ig-h3. This result suggests that the YH motif of β ig-h3 mediates endothelial cell migration through the α v β 3

integrin, and the peptide containing the YH motif inhibits endothelial cell migration by interacting with the $\alpha v \beta 3$ integrin.

Example 8: Assay of angiogenesis inhibition by YH18 synthetic peptide

5 8-1: Endothelial tube formation assay

To examine whether the YH motif in the fas-1 domain inhibits angiogenesis, the present inventors assayed the effect of the peptide on endothelial tube formation.

First, 100 μ l of Matrigel (Chemicon, International Inc, Temecula, CA) was added to each well of a 96-well plate and allowed to polymerize. HUVECs were
10 suspended in medium at a density of 3×10^5 cells/ml, and 0.1 ml of the cell suspension was added to each well of the well plate coated with Matrigel. At this time, 500 μ M or 1 mM of the YH18 synthetic peptide represented by SEQ ID NO: 26 was added together. A control to peptide treatment was treated with 5% DMSO (solvent), and a control to the YH18 synthetic peptide was treated with the YH18-
15 con. peptide represented by SEQ ID NO: 27. Thereafter, the cells were incubated at 37 °C for 16-18 hours. The cells were photographed, and endothelial tubes were counted and averaged.

The results showed that, as shown in Fig. 8a, the YH18 synthetic peptide selectively inhibited the dose-dependent manner. The IC_{50} of the YH18 synthetic
20 peptide was 500 μ M.

8-2: Matrigel Plug assay

In the present invention, the angiogenesis-inhibitory effect of the YH18 synthetic peptide, which had been proven *in vitro* assay according to Example 8-1,
25 was assayed *in vivo*. An *in vivo* Matrigel plug assay was performed according to

the method described by Maeshima, Y. *et al.*, J. Biol. Chem., 275:23745-23750, 2000. 5-6 week old male C57/BL6 mice purchased from Hyochang scientific company, Korea, were used.

First, Matrigel (BD Biosciences, MA) was mixed with 20 units/ml heparin, 150
5 ng/ml bFGF (basic fibroblast growth factor, R&D system, International, Inc), and a YH18 synthetic peptide represented by SEQ ID NO: 26. As a control to the YH18 synthetic peptide, an YH18-con. peptide was used, and a control to peptide treatment was treated with 5% DMSO (solvent). The Matrigel mixture was injected subcutaneously to the C57/BL6 mice. After 7 days, the mice were sacrificed, and
10 the Matrigel plugs were removed and fixed in 4% paraformaldehyde. The Matrigel plugs were buried in paraffin, sectioned and stained with H&E. Their sections were examined by light microscope, the number of blood vessels from 4-6 HPF (high power fields; x200) was counted and averaged. Each group consisted of 3 or 4 Matrigel plugs.

15 The results showed that a significant reduction in the number of blood vessels was observed at 500 μ M of the YH18 synthetic peptide and a complete inhibition of angiogenesis was observed at 1 mM of the peptide (see A of Fig. 8b). Also, the result of observation of the section was the same as described above (see B and C of Fig. 8b).

20

These results suggest that the peptide containing the YH motif inhibits angiogenesis both *in vitro* and *in vivo*.

Application Example 1: Cancers

25 Angiogenesis is an essential stage in the growth and metastasis of cancer

cells (Weidner, N. *et al.*, *N. Engl. J. Med.*, 324:1-8, 1991). Tumors are supplied with nutrients and oxygen necessary for their growth and proliferation through new blood vessels, and also new blood vessels invaded by tumors provides an opportunity for cancer cells to enter the blood circulation, thereby causing the metastasis of the cancer cells (Folkman and Tyler, *Cancer Invasion and Metastasis*, Biologic mechanisms and Therapy (S. B. Day ed.), Raven press, New York, 94-103, 1977; Polverini P. J. *Critical Reviews in Oral Biology*, 6(3):230-247, 1995). If angiogenesis does not occur, the tumors will remain in a resting state and will no longer grow (Folkman and Tyler, *Cancer Invasion and Metastasis*, Biologic mechanisms and Therapy (S. B. Day ed.), Raven press, New York, 94-103, 1977). However, as angiogenesis in cancer tissues develops, cancer cell metastasis toward other tissues occurs (Weidner, N. *et al.*, *N. Engl. J. Med.*, 324:1-8, 1991). The metastasis of cancer cells by blood flow rarely occurs through preexisting blood vessels but mainly occurs at sites where angiogenesis actively occurs. In other words, cancer cells flow out through the incomplete walls of blood vessels, or flows out through the basement membrane of blood vessel walls when the basement membrane is degraded by the action of protease, thereby causing systemic metastasis. In some cases of systemic metastasis, endothelial cells being proliferated cause cancer cells to directly migrate into new blood vessels, thereby causing systemic metastasis. Accordingly, the inventive composition for the inhibition of angiogenesis, which contains the YH motif-containing peptide as an active ingredient, has an excellent angiogenesis inhibitory effect, and thus, is highly effective in the prevention of metastasis and the treatment of various cancers.

Application Example 2: Arthritis

Arthritis is an autoimmune disorder. However, a chronic inflammation, which is formed in the synovial cavity between joints during the progression of arthritis, induces angiogenesis to destroy cartilages. Arthritis includes infectious arthritis, degenerative arthritis, rheumatoid arthritis, and arthritis caused by femoral head avascular necrosis, ankylosing spondylitis and congenital malformation. Regardless of the cause of arthritis, the chronic inflammation formed in the synovial cavity between joints during the progression of arthritis is known to induce angiogenesis. It is characterized in that new capillary vessels invade joint to cause damage to cartilages (Kocb A. E. *et al.*, *Arth. Rheum.*, 29:471-479, 1986; Stupack D. G. *et al.*, *J. Med. Biol. Rcs.*, 32:578-281, 1999; Koch A. E., *Arthritis Rheum.*, 41:951-962, 1998). In this case, it has been reported that an inflammatory response, which occurs in several steps depending the kind of diseases to destroy cartilages, plays an important role in the progression of the disease, and the formation of angiogenesis into joints acts as an important pathological mechanism (Colville-Nash, P.R. *et al.*, *Ann. Rheum. Dis.*, 51, 919-925, 1992; Eisenstein, R., *Pharmacol. Ther.*, 49:1-19, 1991). For the treatment of arthritis, it is preferred to inhibit pains and inflammations so as to reduce the destruction rate of joints or muscles and minimize loss of their function. Accordingly, the inventive composition for the inhibition of angiogenesis is highly effective in the prevention of arthritis progression and in the treatment of arthritis.

Application Example 3: Psoriasis

Psoriasis is a skin disease that involves papules and silver white scars. It is generally a chronic proliferative disorder whose deterioration and improvement are repeated. Also, its cause is not yet identified, but it is known that the formation of

new blood cells on pathological lesions or non-lesions, and also the infiltration of immune cells, such as neutrophil, as a result of an increase in blood vessel permeability, play an important role in the deterioration of psoriasis (Bhushan, M. *et al.*, *Br. J. Dermatol.*, 141:1054-1060, 1999). In normal persons, keratinocytes are proliferated one time a month, but in patients with psoriasis, keratinocytes are proliferated one time a week. Since much blood is necessary for this frequent proliferation, angiogenesis will necessarily occur fast (Folkman J. *J. Invest. Dermatol.*, 59:40-48, 1972). Accordingly, the inventive composition for the inhibition of angiogenesis is effective in the treatment of psoriasis.

Application Example 4: Diabetic eye diseases

Ophthalmic diseases by which several million persons each year in the world lose their sight are also caused by angiogenesis (Jeffrey M. I. *et al.*, *J. Clin. Invest.*, 103:1231-1236, 1999; Stupack D. G. *et al.*, *J. Med. Biol. Res.*, 32:578-281, 1999). Typical examples of the ophthalmic diseases include age-related macular degeneration(AMD), diabetic retinopathy, retinopathy of prematurity, neovascular glaucoma, and corneal diseases caused by angiogenesis (Adamis A. P. *et al.*, *Angiogenesis*, 3:9-14, 1999). Among them, the diabetic eye disease is one of main diabetic complications capable of causing loss of eyesight, and occurs in a patient with long diabetic duration regardless of the regulation of blood glucose. With a recent improvement in diabetic therapy, the lifespan of diabetic patients is extended while diabetic retinopathy shows a tendency to increase. Thus, the diabetic retinopathy is the leading cause of adult blindness in Western Europe and also Korea. The diabetic retinopathy develops due to the functional reduction of retinal circulation so that angiogenesis spreads along the internal surface and posterior

hyaloid membrane of the retina while blood vessels invade the hyaloid, or bleeding occurs, resulting in blindness. Particularly, it has been reported that diabetic eye diseases, such as diabetic retinopathy, are caused by rapid progression of angiogenesis (Favard, C. *et al.*, *Diabetes, Metab.*, 22:268-273, 1996). Accordingly, the inventive composition for the inhibition of angiogenesis is highly effective in the prevention and treatment of diabetic eye diseases.

Application Example 5: Arterial sclerosis

Sclerosis of the arteries means diseases where arterial walls become thicker to lose their elasticity. It is classified into three morphological categories, the most frequent and important category of which is atherosclerosis caused by the formation of atheroma in the arteries. The atheroma, which is formed of cholesterol and cholesterol ester and enclosed in a fibrous membrane, covers the tunica intima of the arteries while the lumen of arterial walls becomes narrower to block the blood flow of distal organs, thereby causing ischemic injury to the organs. If the atheroma is formed in the main artery, it then weakens the arterial walls to cause aneurysm, blood vessel disruption and thrombosis. In this case, it has been reported that the formation of new blood vessel within atheroma (angiogenesis) plays an important role in weakening the blood vessel walls (Hoshiga, M. *et al.*, *Circ. Res.*, 77:1129-1135, 1995; Kahlon, R. *et al.*, *Can. J. Cardiol.*, 8:60-64, 1992; George, S.J., *Curr. Opin. Lipidol.*, 9:413-423, 1998). Accordingly, the inventive composition for the inhibition of angiogenesis is highly effective in the prevention of severe complications that can be caused by arterial sclerosis.

Application Example 6: Inflammation

Inflammation, which is a response of a living tissue to injury, can be caused by various factors, such as infection and trauma, but show substantially similar changes regardless of its cause and response tissue. Such changes include an increase in blood flow, an increase in permeability of blood vessel walls, and the
5 infiltration of white blood cells, in which all the changes are known to be caused by angiogenesis (Jackson, J. R. *et al.*, *FASEB, J.*, 11:457-465, 1997). Although inflammation is a repairing mechanism of injury and thus not a harmful response, it can cause the injury and deformation of tissues when it excessively occurs or inappropriately occurs as in autoimmune diseases. In regulating such an excessive
10 or inappropriate inflammatory response, the inventive composition for the inhibition of angiogenesis is effective.

INDUSTRIAL APPLICABILITY

As described above, the peptide according to the present invention interacts
15 with the $\alpha v \beta 3$ integrin of endothelial cells, so that it inhibits the adhesion and migration of endothelial cells and also shows an excellent angiogenesis-inhibitory effect. Accordingly, the inventive peptide is useful for the inhibition of endothelial cell adhesion, endothelial cell migration, and/or angiogenesis. In addition, it is useful for the treatment or prevention of various angiogenesis-related diseases.

WHAT IS CLAIMED IS:

1. A method for inhibiting endothelial cell adhesion, endothelial cell migration and/or angiogenesis, comprising administering to a subject in need thereof an effective amount of:

5 (a) an isolated peptide consisting of at least 18 amino acids, comprising tyrosine-histidine (Y-H) or asparagine-histidine (N-H), and at least three hydrophobic amino acids with bulky side chains; or

(b) a mutant or derivative of the peptide (a), in which the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) in the peptide (a) was substituted with amino
10 acids selected from the group consisting of serine-histidine (S-H), histidine-histidine (H-H), phenylalanine-histidine (F-H), threonine-histidine (T-H), tyrosine-asparagine (Y-N) and alanine-alanine (A-A).

2. The method of Claim 1, wherein the hydrophobic amino acids with bulky
15 side chains are isoleucines (I) or leucines (L).

3. The method of Claim 1, wherein the peptide comprises an amino acid sequence represented by (I, D, E or K)-(E, A or Q)-L-(L, R or A)-(N, D or S)-(A, L, K or I)-(L or Y)-(R, N, L or K)-(Y or N)-H-(M, I or G)-(V, L, Q or G)-(G, K, T or
20 D)-(R, S, L or E)-(R, A, E or I)-(V, M, T or L)-(L, C or V)-(T, A, G or S).

4. The method of Claim 3, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 23 to SEQ ID NO: 26.

5. The method of Claim 1, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17 to SEQ ID NO: 22.

6. The method of Claim 5, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11 to SEQ ID NO: 16.

7. A method for the treatment or prevention of angiogenesis-related diseases, comprising administering to a subject in need thereof an effective amount of:

(a) an isolated peptide consisting of at least 18 amino acids, comprising tyrosine-histidine (Y-H) or asparagine-histidine (N-H), and at least three hydrophobic amino acids with bulky side chains; or

(b) a mutant or derivative of the peptide (a), in which the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) in the peptide (a) was substituted with amino acids selected from the group consisting of serine-histidine (S-H), histidine-histidine (H-H), phenylalanine-histidine (F-H), threonine-histidine (T-H), tyrosine-asparagine (Y-N) and alanine-alanine (A-A).

8. The method of Claim 7, the angiogenesis-related diseases are selected from the group consisting of cancer, vascular malformation, arteriosclerosis, vascular adhesions, edematous sclerosis, corneal graft neovascularization, neovascular glaucoma, diabetic retinopathy, pterygium, retinal degeneration, retrolental fibroplasia, granular conjunctivitis, rheumatoid arthritis, systemic Lupus erythematosus, thyroiditis, psoriasis, capillarectasia, pyogenic granuloma, seborrheic dermatitis and acne.

25

9. A pharmaceutical composition for the inhibition of angiogenesis, which comprises the following peptide (a) or (b) as an active ingredient:

(a) an isolated peptide consisting of at least 18 amino acids, comprising tyrosine-histidine (Y-H) or asparagine-histidine (N-H), and at least three
5 hydrophobic amino acids with bulky side chains; or

(b) a mutant or derivative of the peptide (a), in which the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) in the peptide (a) was substituted with amino acids selected from the group consisting of serine-histidine (S-H), histidine-histidine (H-H), phenylalanine-histidine (F-H), threonine-histidine (T-H), tyrosine-asparagine
10 (Y-N) and alanine-alanine (A-A).

10. A pharmaceutical composition for the treatment or prevention of angiogenesis-related diseases, which comprises the following peptide (a) or (b) as an active ingredient:

15 (a) an isolated peptide consisting of at least 18 amino acids, comprising tyrosine-histidine (Y-H) or asparagine-histidine (N-H), and at least three hydrophobic amino acids with bulky side chains; or

(b) a mutant or derivative of the peptide (a), in which the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) in the peptide (a) was substituted with amino
20 acids selected from the group consisting of serine-histidine (S-H), histidine-histidine (H-H), phenylalanine-histidine (F-H), threonine-histidine (T-H), tyrosine-asparagine (Y-N) and alanine-alanine (A-A).

11. Use of the following peptide (a) or (b) for the preparation of a pharmaceutical agent for the inhibition of endothelial cell adhesion, endothelial cell migration and/or angiogenesis:

(a) an isolated peptide consisting of at least 18 amino acids, comprising
5 tyrosine-histidine (Y-H) or asparagine-histidine (N-H), and at least three hydrophobic amino acids with bulky side chains; or

(b) a mutant or derivative of the peptide (a), in which the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) in the peptide (a) was substituted with amino acids selected from the group consisting of serine-histidine (S-H), histidine-histidine
10 (H-H), phenylalanine-histidine (F-H), threonine-histidine (T-H), tyrosine-asparagine (Y-N) and alanine-alanine (A-A).

12. Use of the following peptide (a) or (b) for the preparation of an agent for the treatment or prevention of angiogenesis-related diseases:

15 (a) an isolated peptide consisting of at least 18 amino acids, comprising tyrosine-histidine (Y-H) or asparagine-histidine (N-H), and at least three hydrophobic amino acids with bulky side chains; or

(b) a mutant or derivative of the peptide (a), in which the tyrosine-histidine (Y-H) or asparagine-histidine (N-H) in the peptide (a) was substituted with amino
20 acids selected from the group consisting of serine-histidine (S-H), histidine-histidine (H-H), phenylalanine-histidine (F-H), threonine-histidine (T-H), tyrosine-asparagine (Y-N) and alanine-alanine (A-A).

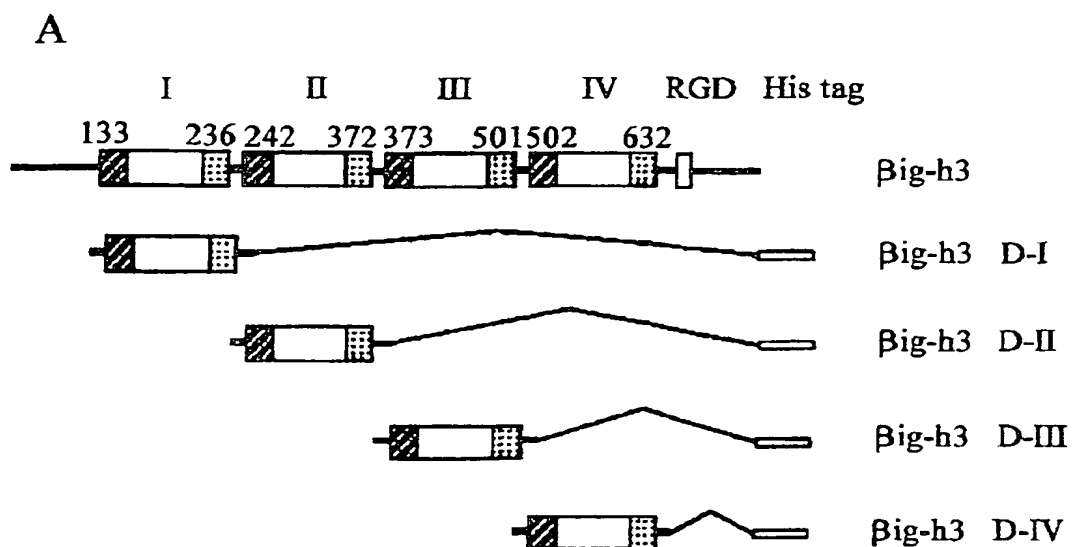
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FIG. 1

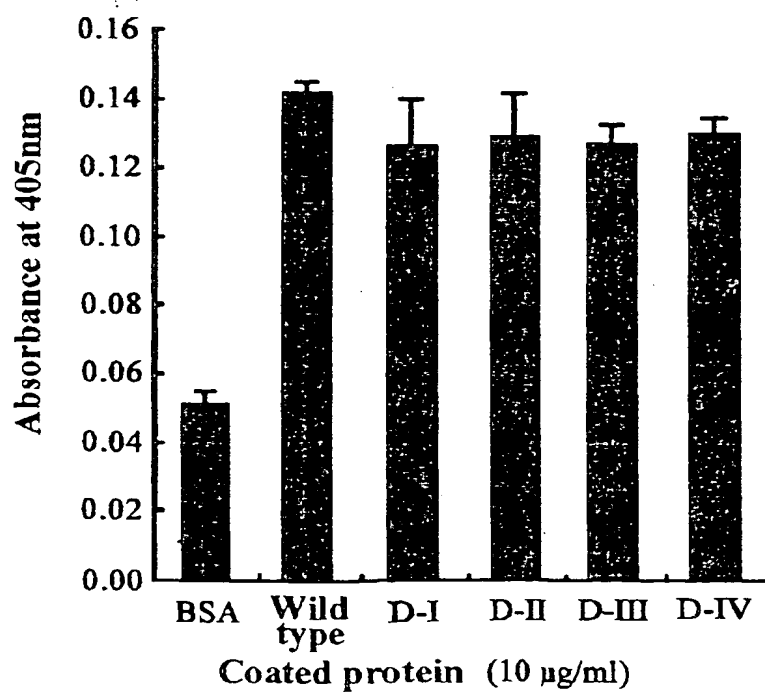
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BIGH3_CHICK	154	SNVNIETINADRYH	MVNKRV	TDD	KHGTT	183
OSF2_MOUSE	157	NNVNVEETINADHSH	MVNKRN	TKD	KHGMV	186
BIGH3_HUMAN	298	-GDPEAPRIENNNH	IKSAMCAEATVAGLS			326
BIGH3-PIG	298	-GDPEAPRIENNNH	IKSAMCAEATVAGLS			326
BIGH3_CHICK	290	-GDPEAPRIENNNH	IKSAMCAEATVAGLT			318
OSF2_HUMAN	292	-GDKVASEAMKRYH	INTIQCSEATGGAV			320
OSF2_MOUSE	294	-GDKVASEAMKRYH	INTIQCSEATGGAV			322
BIGH3_HUMAN	560	-GDAKEIANIKRYH	VGDEIVSGGIGALVR			588
BIGH3-PIG	560	-GNAKEIANIKRYH	VGDEIVSGGIGALVR			588
BIGH3_CHICK	552	-GNAKEIASIKRFH	MADEIVSGAVSALVR			580
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SLL1735	59	-QNIPQAFETTYH	VVAGKETQADICRIST			87
SLL1483	104	PENKDKVVEETTYH	VVPGKITAAQV-QSGE			132
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OSF2_MOUSE	556	-GDKNAIQNITTYH	ETPGVYIGKGFEPGVT			584
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MPT83	145	-TDARLSSSETTYH	VVAGQ--ASPSRIQGT			171
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Q50769_MYCTU	119	-TNSSLETSETTYH	VVAGQ--TSPANVVGT			145
Putative Secreted protein	132	-NDRAQPKKVTTYH	VVEHKRITKAQEPHGT			160
Fasciclin	485	EG-RGCASMTKRNH	MDLTFCSTATVPGAK			513
HLC-32	284	KDPAGKRNITKRYH	VSDVKYSVSSGQR			313
Fasciclin	775	-SKPADPMETVKTHT	IVEDVVCCAGIPTNW			803
BIGH3_HUMAN	432	RNLEIR.NHIVKDOASKY	YH GQTIDTIGGKQIR			464
BIGH3_PIG	432	RNLEIR.NHIVKDOASKY	YH GQTIDTIGGKQIR			464
BIGH3_CHICK	424	RNLEIR.NHIVKDOSSKY	YH GQELQTIQDKQIR			456
OSF2_HUMAN	426	-RLILQNHIIKVKVGLN	LYN GQELQTIQDKQIR			458
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FIG. 2

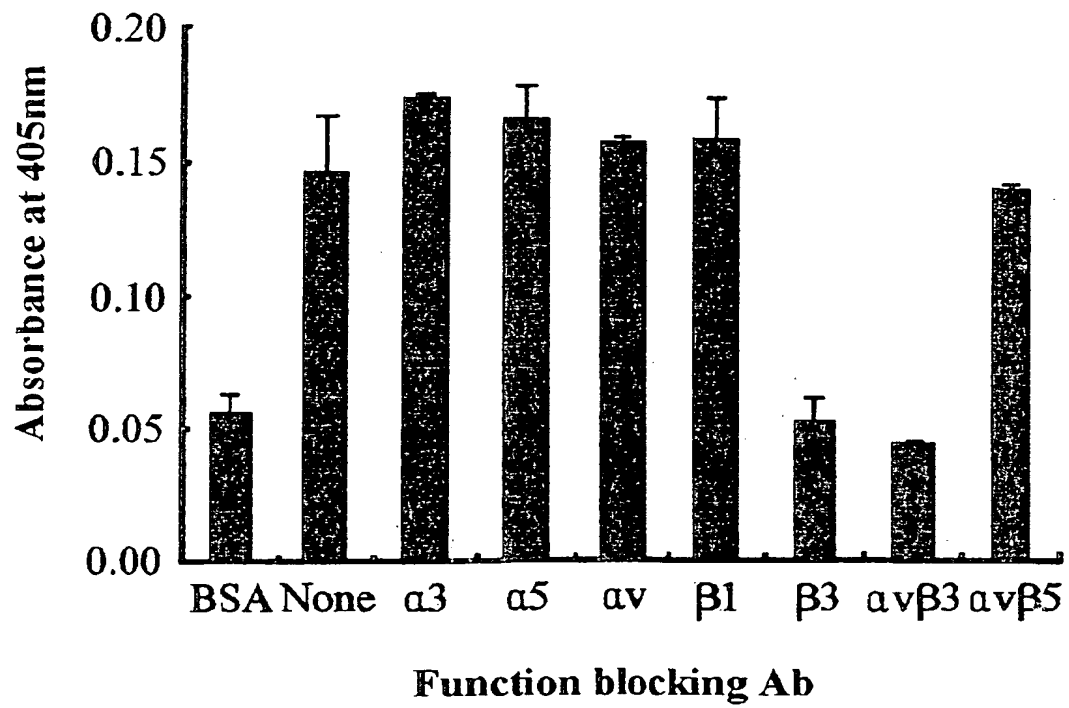


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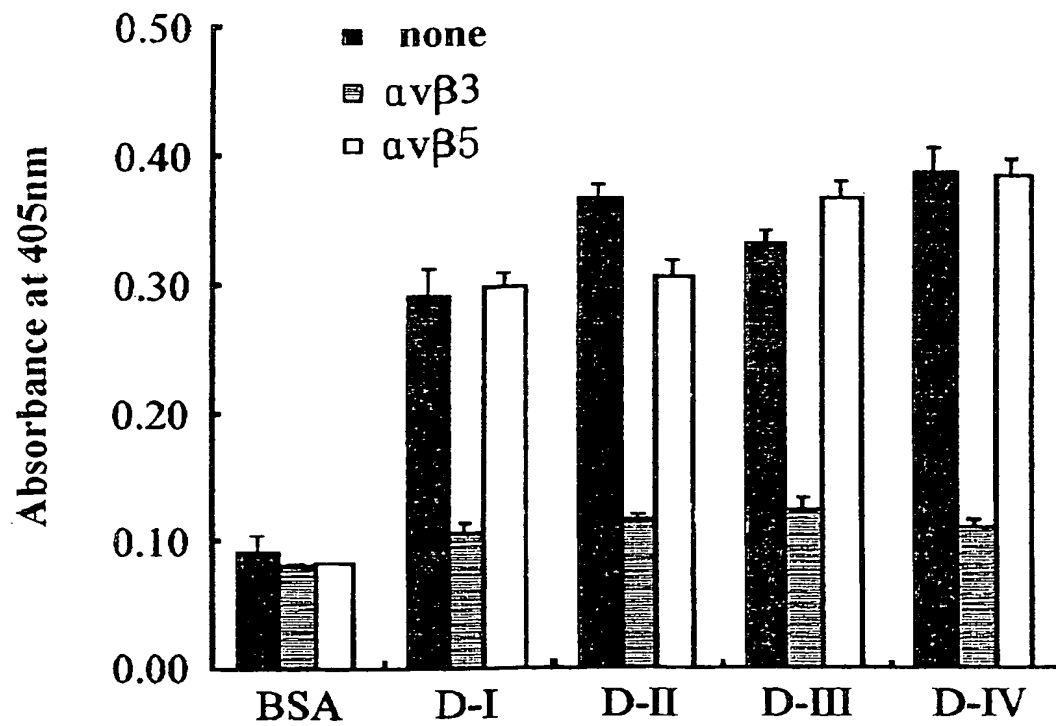
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FIG. 3a



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FIG. 3b



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FIG. 3c

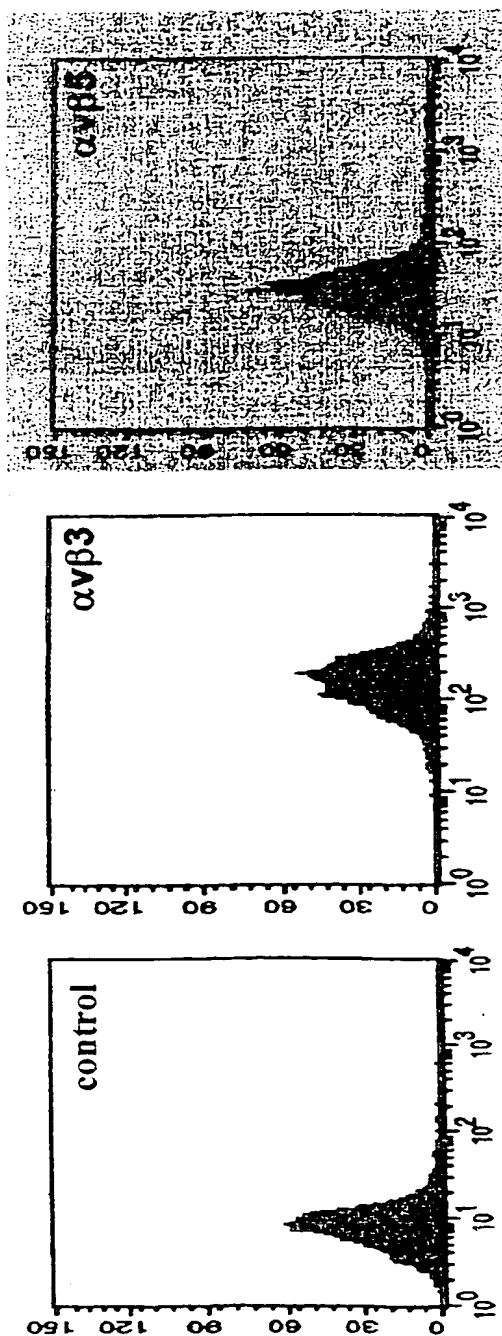
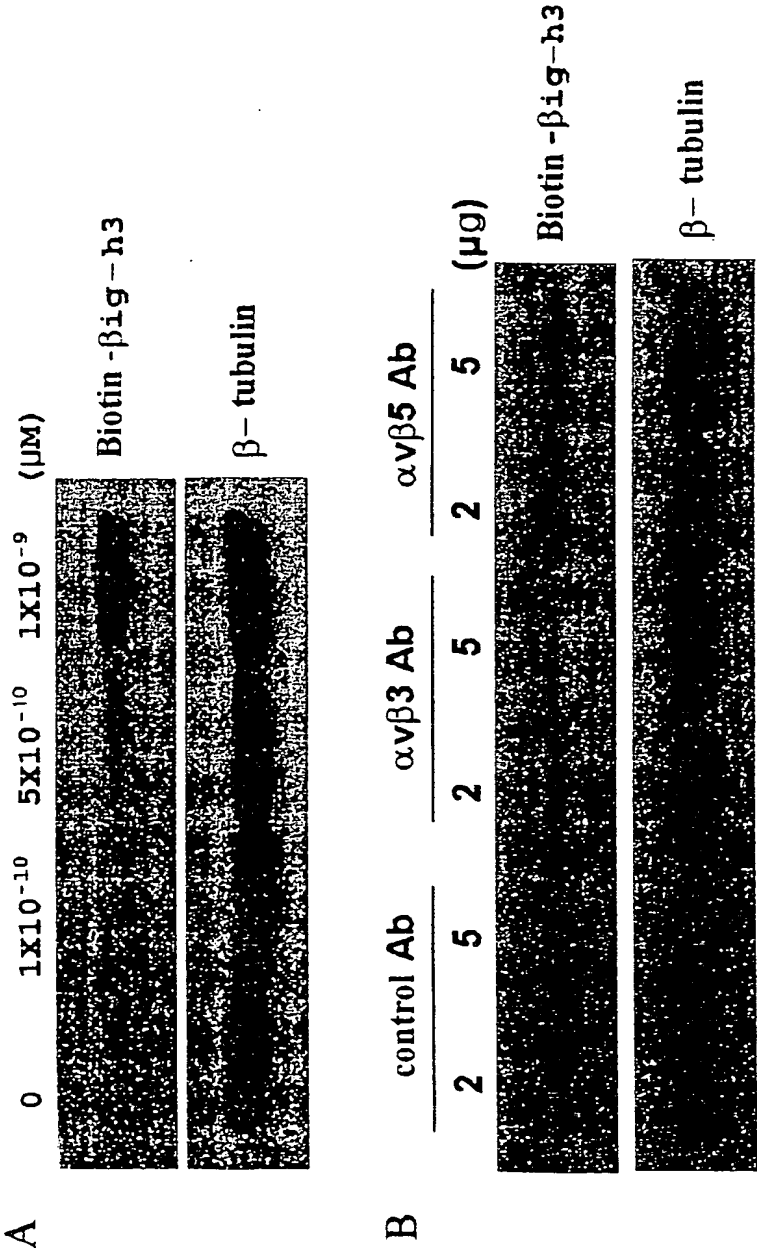


FIG. 3d



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FIG. 4a

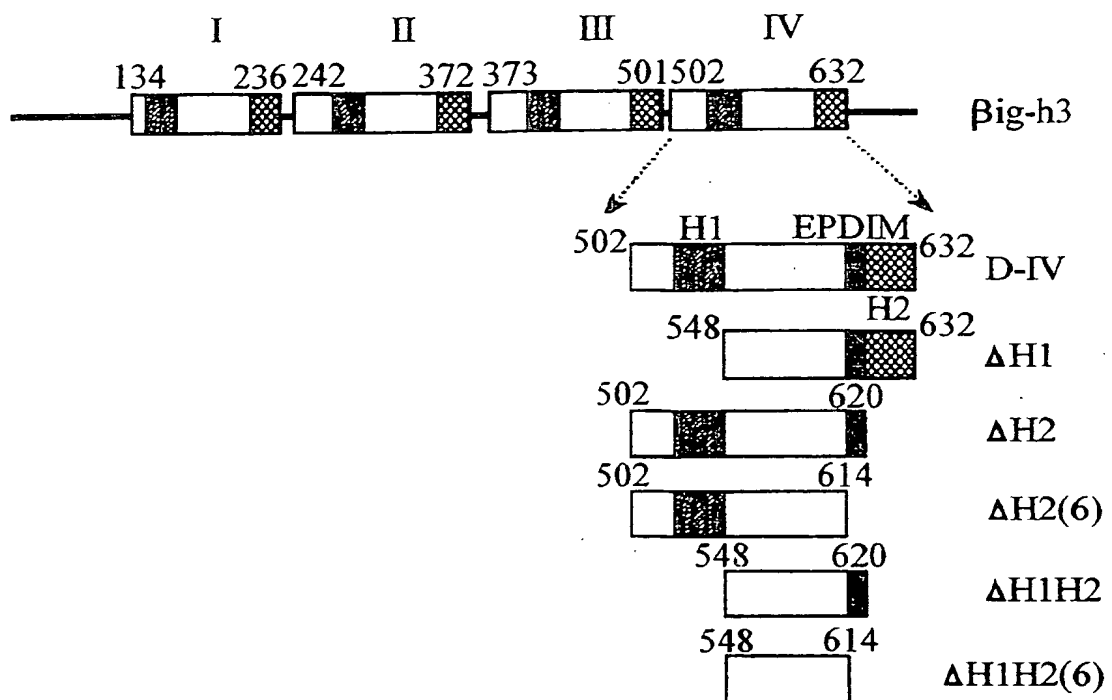
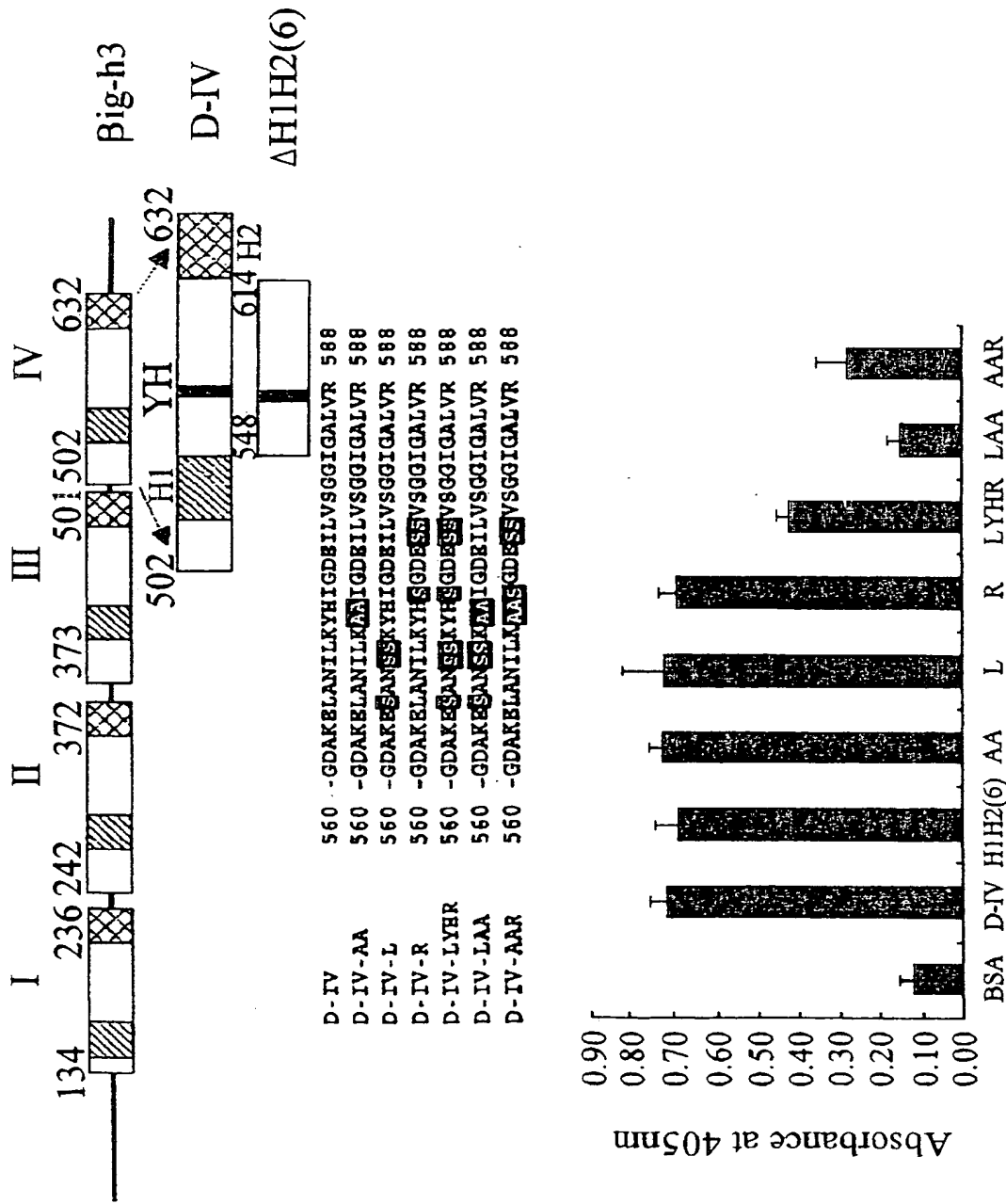


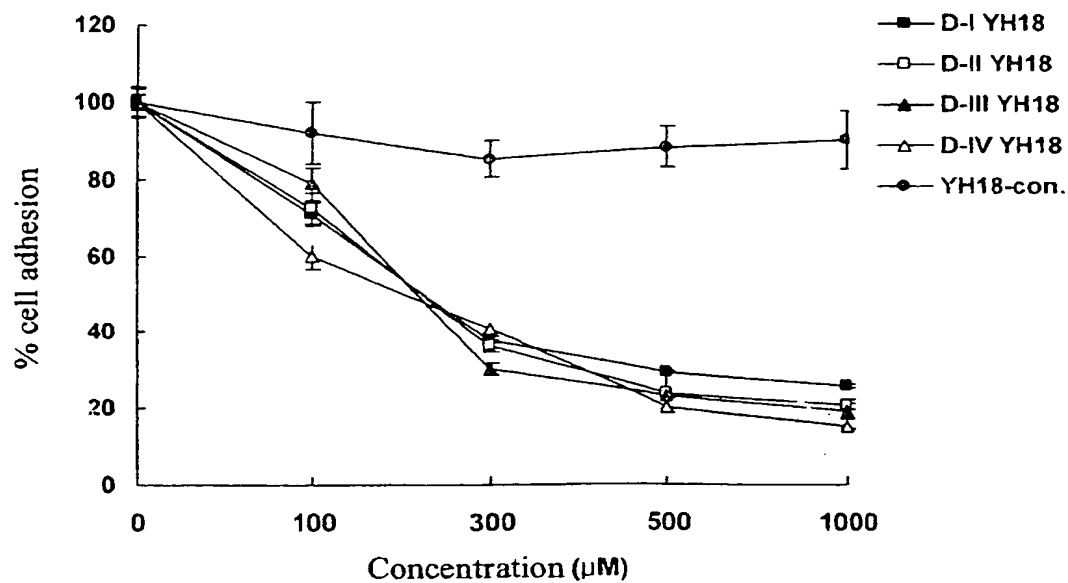
FIG. 4b



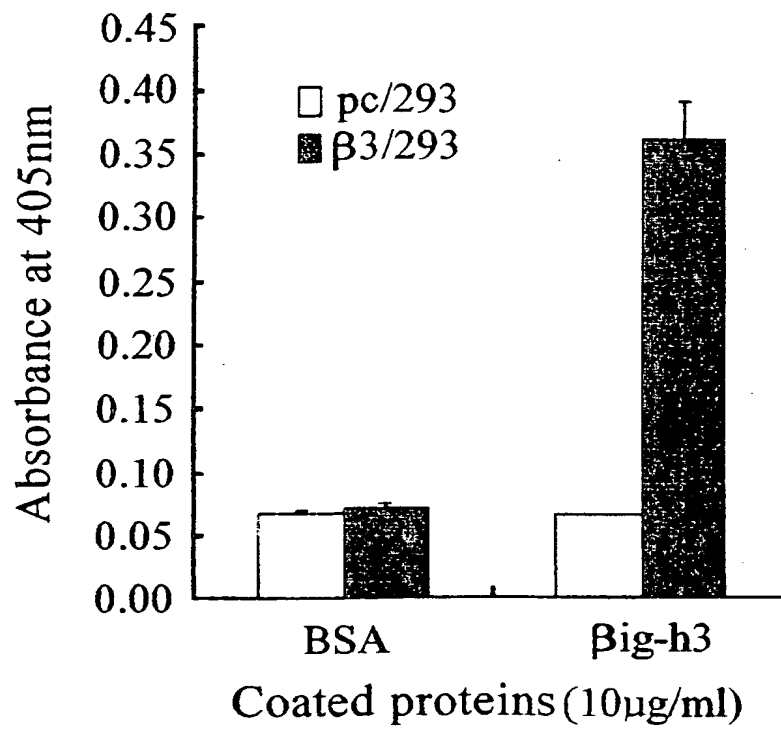
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FIG. 5

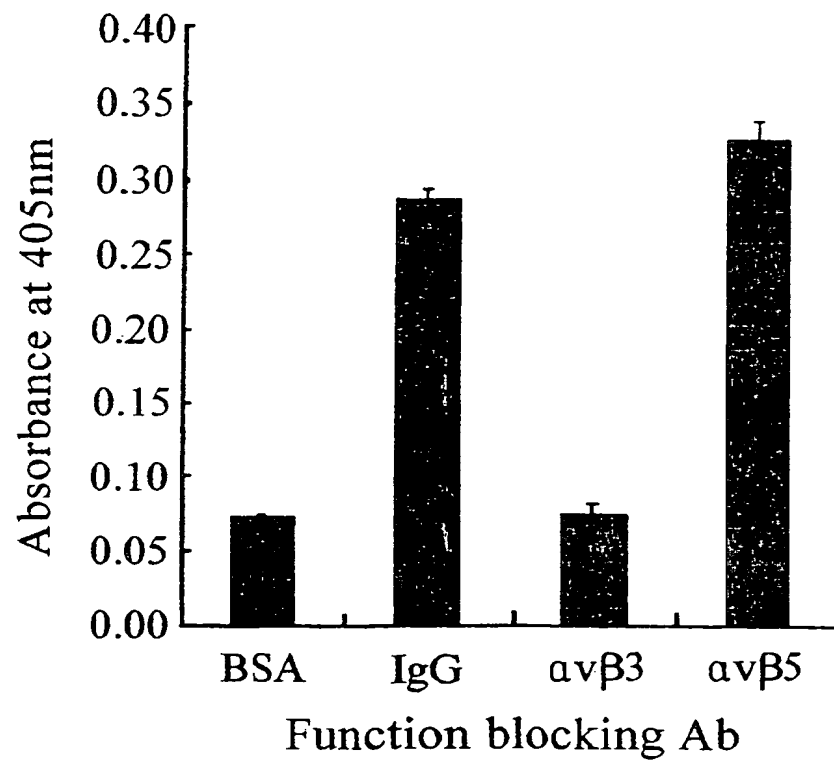
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D-III	YH18	442	DQLASKYLYHGQTLETLG	459
D-IV	YH18	563	KELANILKYHIGDEILVS	580
YH18-con			KELANIHGKLYDEILVS	



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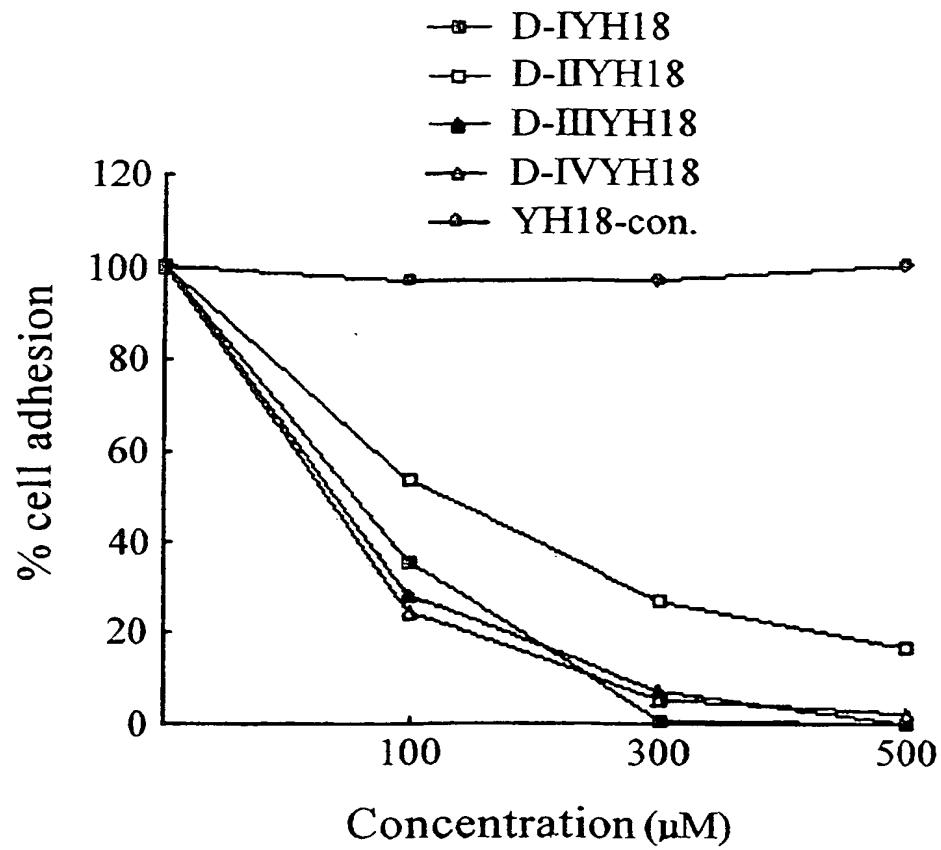
FIG. 6a

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FIG. 6b

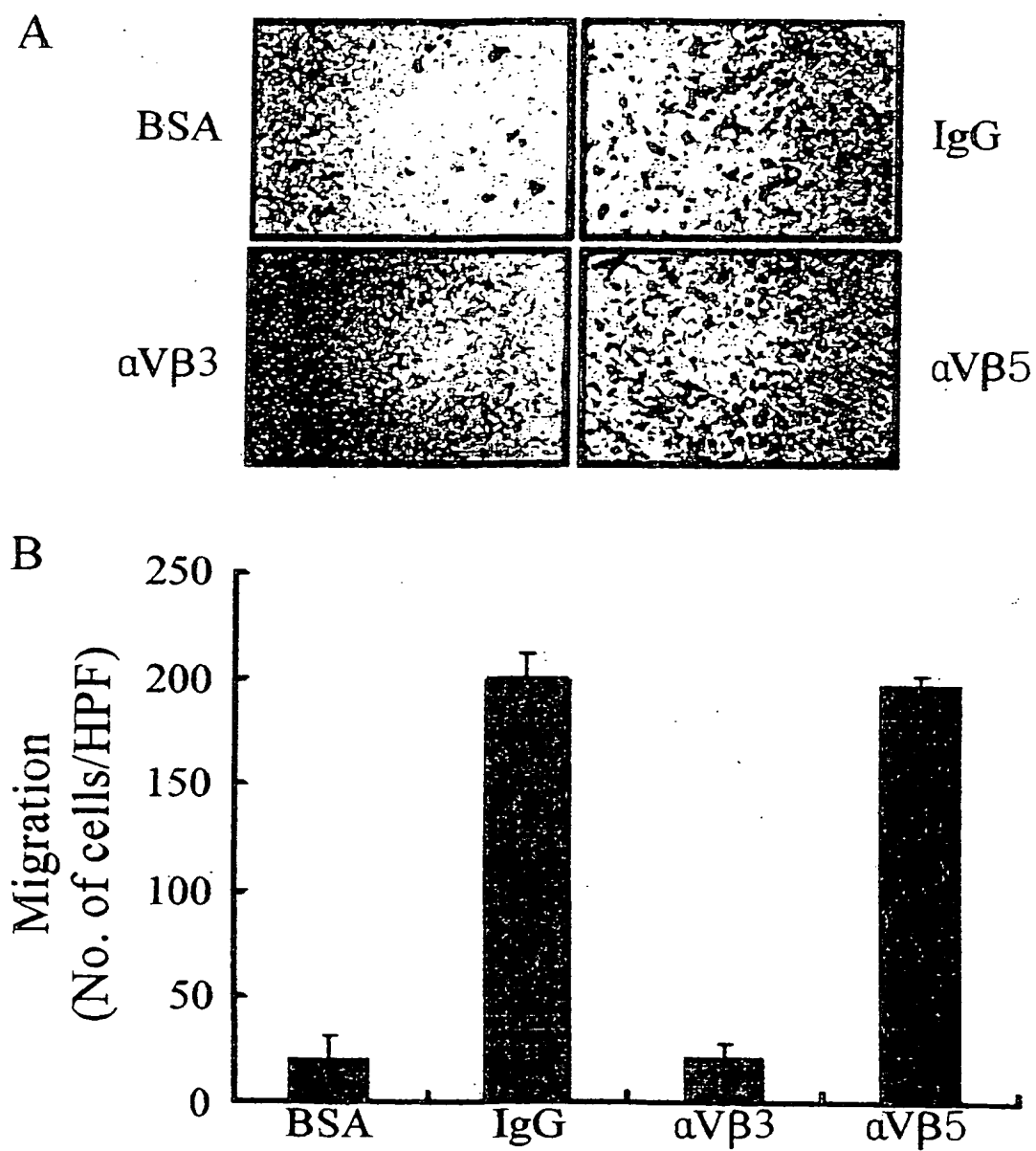
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FIG. 6c



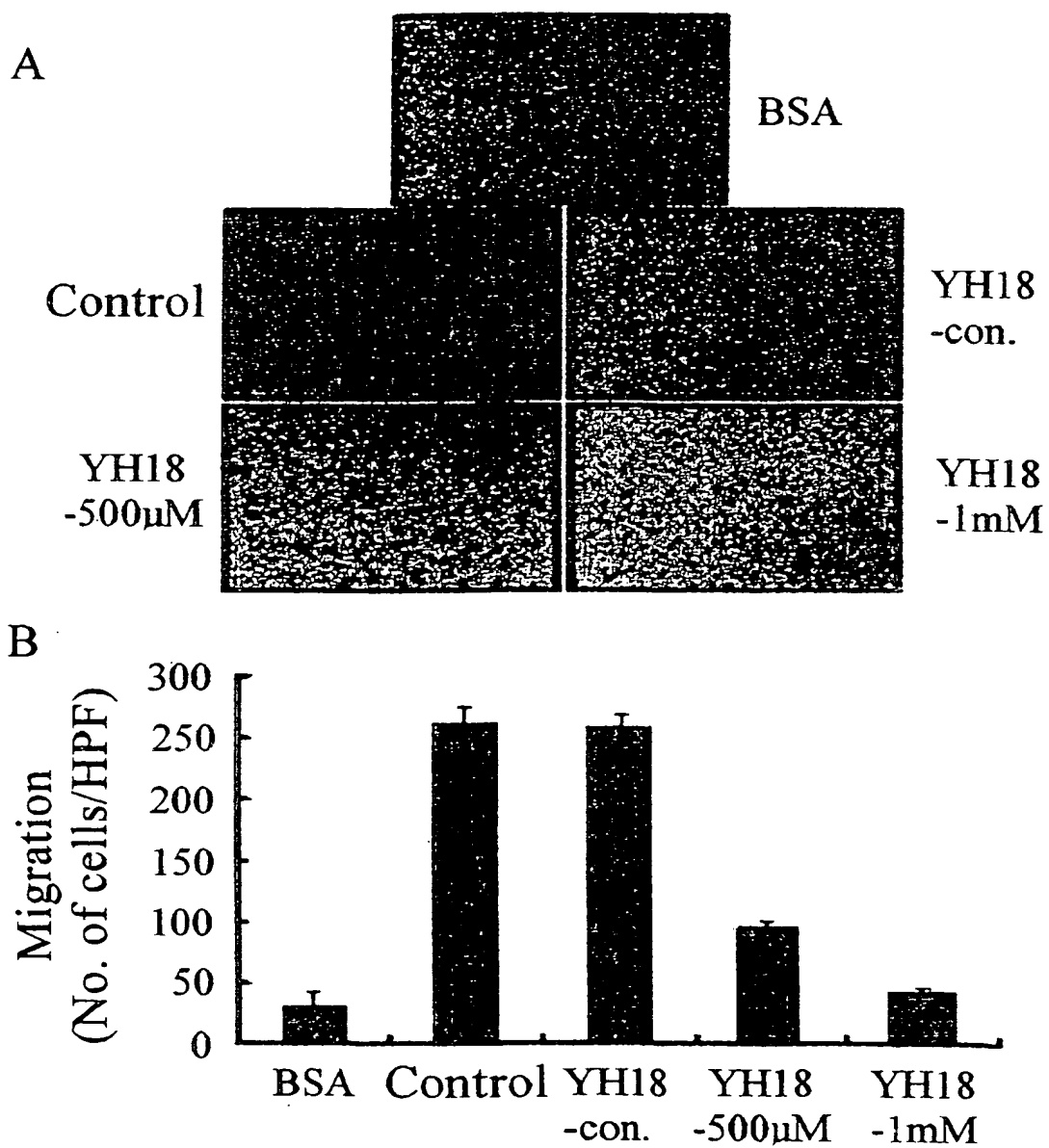
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FIG. 7a



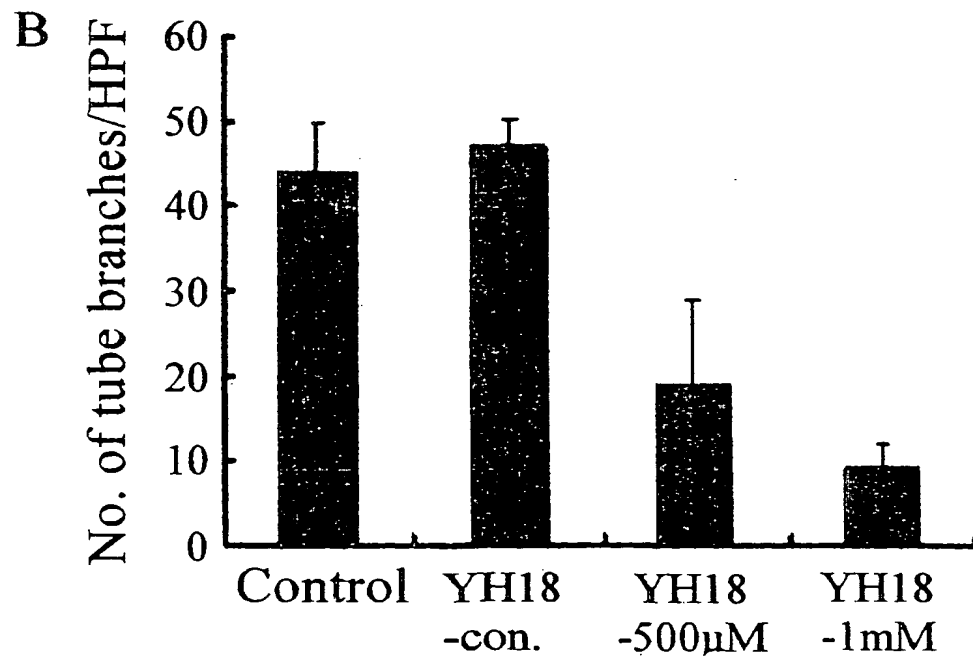
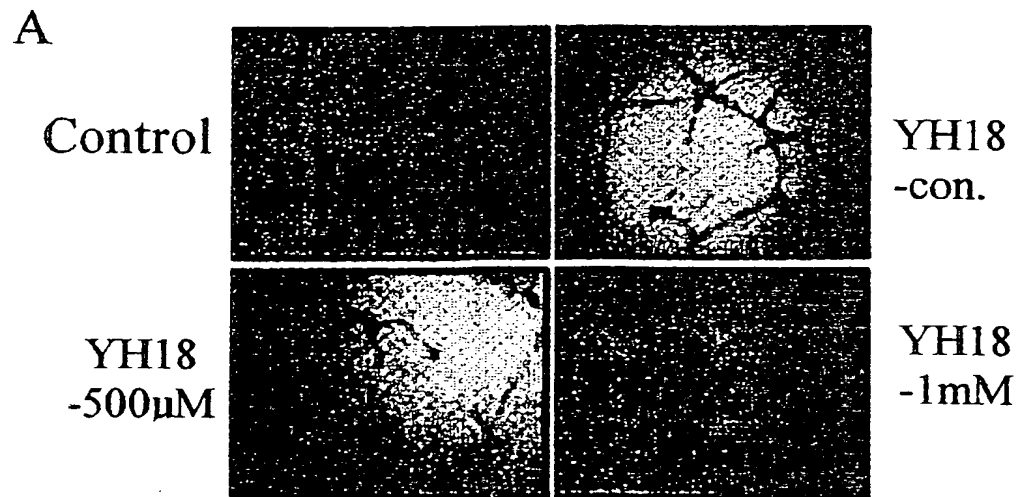
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FIG. 7b



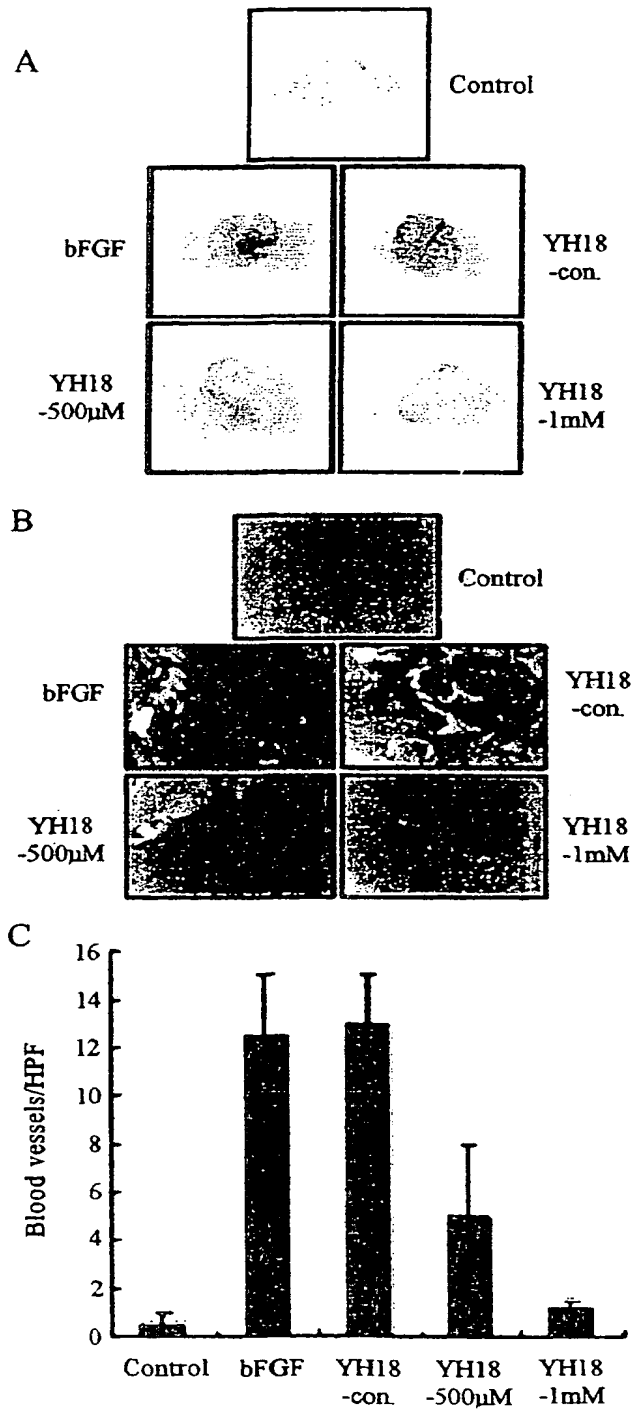
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FIG. 8a



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FIG. 8b



JC20 Rec'd PCT/PTO 03 OCT 2009

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35 40 45

Cys Ala Val Gln Lys Val Ile Gly Thr Asn Arg Lys Tyr Phe Thr Asn
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Cys Lys Gln Trp Tyr Gln Arg Lys Ile Cys Gly Lys Ser Thr Val Ile
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Cys Pro Ala Ala Leu Pro Leu Ser Asn Leu Tyr Glu Thr Leu Gly Val
100 105 110

Val Gly Ser Thr Thr Thr Gln Leu Tyr Thr Asp Arg Thr Glu Lys Leu
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 420 425 430

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<210> 2
 <211> 103
 <212> PRT

<213> Homo sapiens

<400> 2

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 Ser Leu Pro Ala Glu Val Leu Asp Ser Leu Val Ser Asn Val Asn Ile
 20 25 30
 Glu Leu Leu Asn Ala Leu Arg Tyr His Met Val Gly Arg Arg Val Leu
 35 40 45
 Thr Asp Glu Leu Lys His Gly Met Thr Leu Thr Ser Met Tyr Gln Asn
 50 55 60
 Ser Asn Ile Gln Ile His His Tyr Pro Asn Gly Ile Val Thr Val Asn
 65 70 75 80
 Cys Ala Arg Leu Leu Lys Ala Asp His His Ala Thr Asn Gly Val Val
 85 90 95
 His Leu Ile Asp Lys Val Ile
 100

<210> 3

<211> 131

<212> PRT

<213> Homo sapiens

<400> 3

Asn Ile Gln Gln Ile Ile Glu Ile Glu Asp Thr Phe Glu Thr Leu Arg
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 Ala Ala Val Ala Ala Ser Gly Leu Asn Thr Met Leu Glu Gly Asn Gly
 20 25 30
 Gln Tyr Thr Leu Leu Ala Pro Thr Asn Glu Ala Phe Glu Lys Ile Pro
 35 40 45
 Ser Glu Thr Leu Asn Arg Ile Leu Gly Asp Pro Glu Ala Leu Arg Asp
 50 55 60
 Leu Leu Asn Asn His Ile Leu Lys Ser Ala Met Cys Ala Glu Ala Ile
 65 70 75 80
 Val Ala Gly Leu Ser Val Glu Thr Leu Glu Gly Thr Thr Leu Glu Val
 85 90 95
 Gly Cys Ser Gly Asp Met Leu Thr Ile Asn Gly Lys Ala Ile Ile Ser
 100 105 110

Asn Lys Asp Ile Leu Ala Thr Asn Gly Val Ile His Tyr Ile Asp Glu
 115 120 125

Leu Leu Ile
 130

<210> 4
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 <212> PRT
 <213> Homo sapiens

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 Pro Asp Ser Ala Lys Thr Leu Phe Glu Leu Ala Ala Glu Ser Asp Val
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 20 25 30

Ser Gly Ser Glu Arg Leu Thr Leu Leu Ala Pro Leu Asn Ser Val Phe
 35 40 45

Lys Asp Gly Thr Pro Pro Ile Asp Ala His Thr Arg Asn Leu Leu Arg
 50 55 60

Asn His Ile Ile Lys Asp Gln Leu Ala Ser Lys Tyr Leu Tyr His Gly
 65 70 75 80

Gln Thr Leu Glu Thr Leu Gly Gly Lys Lys Leu Arg Val Phe Val Tyr
 85 90 95

Arg Asn Ser Leu Cys Ile Glu Asn Ser Cys Ile Ala Ala His Asp Lys
 100 105 110

Arg Gly Arg Tyr Gly Thr Leu Phe Thr Met Asp Arg Val Leu Thr Pro
 115 120 125

Pro

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Leu Val Ala Ala Ile Gln Ser Ala Gly Leu Thr Glu Thr Leu Asn Arg
 20 25 30

Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala
 35 40 45
 Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu
 50 55 60
 Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly
 65 70 75 80
 Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu
 85 90 95
 Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn Lys Glu Pro Val
 100 105 110
 Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val His Val Ile Thr
 115 120 125
 Asn Val Leu
 130

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 Ser Gly Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp
 35 40 45
 Lys Leu Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn Lys Glu
 50 55 60
 Pro Val Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val His Val
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 Ile Thr Asn Val Leu
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 <211> 119
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 20 25 30

Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala
 35 40 45

Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu
 50 55 60

Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly
 65 70 75 80

Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu
 85 90 95

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 100 105 110

Ala Glu Pro Asp Ile Met Ala
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<211> 113

<212> PRT

<213> Homo sapiens

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 20 25 30

Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala
 35 40 45

Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu
 50 55 60

Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly
 65 70 75 80

Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu
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Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn Lys Glu Pro Val

100 105 110

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 <211> 73
 <212> PRT
 <213> Homo sapiens

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 20 25 30
 Ser Gly Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp
 35 40 45
 Lys Leu Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn Lys Glu
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 Pro Val Ala Glu Pro Asp Ile Met Ala
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 <211> 67
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 Glu Leu Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile Leu Val
 20 25 30
 Ser Gly Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp
 35 40 45
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 50 55 60
 Pro Val Ala
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<212> PRT
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Val Ser

<210> 12
 <211> 18
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Val Ser

<210> 13
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Val Ser

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Val Ser

<210> 15

<211> 18

<212> PRT

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<211> 18

<212> PRT

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Val Ser

<210> 17

<211> 29

<212> PRT

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<223> D-IV-AA

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<211> 29

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<220>

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Glu Ile Leu Val Ser Gly Gly Ile Gly Ala Leu Val Arg
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<210> 19

<211> 29

<212> PRT

<213> Artificial Sequence

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<223> D-IV-R

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Glu Ser Ser Val Ser Gly Gly Ile Gly Ala Leu Val Arg
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<223> D-IV-LYHR

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Glu Ser Ser Val Ser Gly Gly Ile Gly Ala Leu Val Arg
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Glu Ile Leu Val Ser Gly Gly Ile Gly Ala Leu Val Arg
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<210> 22

<211> 29

<212> PRT

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<223> D-IV-AAR

<400> 22

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Glu Ser Ser Val Ser Gly Gly Ile Gly Ala Leu Val Arg
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<210> 23

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<220>

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Leu Thr

<210> 24

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

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<212> PRT

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<220>

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Leu Gly

<210> 26

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> D-IV YH18

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Val Ser

<210> 27

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> YH18-con.

<400> 27

Lys Glu Leu Ala Asn Ile His Gly Ile Lys Leu Tyr Asp Glu Ile Leu
1 5 10 15

Val Ser

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2004/000774

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 A61K 38/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NCBI protein blast program

Pubmed [(betaig-h3 AND alphavbeta3 intergrin) OR (alphavbeta3integrin AND angiogenesis)]

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	NAM et al., 'Identification of the alphavbeta3 integrin-interacting motif of betaig-h3 and its anti-angiogenic effect', The Journal of Biological Chemistry, July 2003, Vol.278, No.28, pp.25902-25909 See abstract.	1 - 12
A	HORTON, 'The alphavbeta3 integrin "Vitronectin Receptor"', International Journal of Biochemistry and Cell Biology, 1997, Vol.29, No.5, pp.721-725 See abstract.	1 - 12
A	KIM et al., 'Identification of motifs in the fasciclin domains of the transforming growth factor-beta-induced matrix protein betaig-h3 that interact with the alphavbeta5 integrin', The Journal of Biological Chemistry, Nov 2002, Vol.277, No.48, pp.46159-46165 See abstract.	1 - 12
A	KIM et al., 'Identification of motifs for cell adhesion within the repeated domains of transforming growth factor-beta-induced gene, betaig-h3', The Journal of Biological Chemistry, Oct 2000, Vol.275, No.40, pp.30907-30915 See abstract.	1 - 12

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family


Date of the actual completion of the international search

14 JUNE 2004 (14.06.2004)

Date of mailing of the international search report

15 JUNE 2004 (15.06.2004)

Name and mailing address of the ISA/KR

 Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

LEE, Mi Jeong

Telephone No. 82-42-481-5601



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2004/000774

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of :

a. type of material

- ☒ a sequence listing
☐ table(s) related to the sequence listing

b. format of material

- ☒ in written format
☒ in computer readable form

c. time of filing/furnishing

- ☒ contained in the international application as filed
☒ filed together with the international application in computer readable form
☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: